10/790,589

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     (FILE 'HOME' ENTERED AT 09:06:25 ON 04 NOV 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004
L1          1382 S "DEOXYRIBONUCLEASE II"
L2           377 S L1 AND (HUMAN OR MURINE)
L3       3858984 S BETA
L4            36 S L2 AND L3
L5            27 DUP REM L4 (9 DUPLICATES REMOVED)
L6       1714908 S DIGEST?
L7           348 S L1 AND L6
L8            76 S L7 AND (HUMAN OR MURINE)
L9            41 DUP REM L8 (35 DUPLICATES REMOVED)
                 E EASTMAN A R/AU
L10           21 S E3
                 E KRIESER R J/AU
L11           79 S E3-E8
L12           99 S L10 OR L11
L13           57 S L1 AND L12
L14           15 DUP REM L13 (42 DUPLICATES REMOVED)

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NEWS	13	SEP 27	SWETSCAN will no longer be available on STN
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NEWS EXPRESS	OCTOBER 29 CURRENT WINDOWS VERSION IS V7.01A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
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NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

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=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
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	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.84	0.84

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FILE 'LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)

=> s "deoxyribonuclease II"
L1 1382 "DEOXYRIBONUCLEASE II"

=> s l1 and (human or murine)
4 FILES SEARCHED...
L2 377 L1 AND (HUMAN OR MURINE)

=> s beta
L3 3858984 BETA

=> s l2 and l3
L4 36 L2 AND L3

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 27 DUP REM L4 (9 DUPLICATES REMOVED)

=> d 1-27 ibib ab

L5 ANSWER 1 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:780908 HCAPLUS
DOCUMENT NUMBER: 141:295286
TITLE: Biomarker identification for evaluating caloric restricted diet program in mammals
INVENTOR(S): Spindler, Stephen R.; Dhahbi, Joseph M.
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 113 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004081537	A2	20040923	WO 2004-US7737	20040312

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2004180003	A1	20040916	US 2003-387743	20030312
US 2004191775	A1	20040930	US 2003-387786	20030312
PRIORITY APPLN. INFO.:			US 2003-387743	A 20030312
			US 2003-387786	A 20030312
			US 2003-622160	A 20030716

AB Methods of identifying biomarkers of calorie restriction and of examining the dynamics of calorie restriction are presented. In addition, the invention provides methods of selecting mimetics of calorie restriction.

L5 ANSWER 2 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:718660 HCAPLUS
DOCUMENT NUMBER: 141:237741
TITLE: Production of modified glycoproteins having multiple antennary structures by expression of glucosaminyltransferases in fungal cells
INVENTOR(S): Bobrowicz, Piotr; Hamilton, Stephen R.; Gerngross, Tilman U.; Wildt, Stefan; Choi, Byung-Kwon; Nett, Juergen Hermann; Davidson, Robert C.
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 231 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004074461	A2	20040902	WO 2004-US5191	20040220

W: AE, AE, AG, AL, AL, AM, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG, BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR, CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES, ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN, IS, JP, JP, KE, KE, KG, KG, KP, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2004018590	A1	20040129	US 2003-371877	20030220
PRIORITY APPLN. INFO.:			US 2003-371877	A 20030220
			US 2003-680963	A 20031007
			US 2000-214358P	P 20000628
			US 2000-215638P	P 20000630
			US 2001-279997P	P 20010330
			US 2001-892591	A2 20010627

AB The present invention relates to eukaryotic host cells, especially lower eukaryotic host cells, having modified oligosaccharides which may be modified further by heterologous expression of a set of glycosyltransferases, sugar and sugar nucleotide transporters to become host-strains for the production of mammalian, e.g., **human**

therapeutic glycoproteins. The process provides an engineered host cell which can be used to express and target any desirable gene(s) involved in glycosylation. Host cells with modified lipid-linked oligosaccharides are created or selected. N-glycans made in the engineered host cells are substrates for GnTIII, GnTIV, GnTV, GnT VI or GnTIX activity, which produce bisected and/or multiantennary N-glycan structures and may be modified further by heterologous expression of one or more enzymes, e.g., glycosyltransferases, sugar, sugar nucleotide transporters, to yield **human**-like glycoproteins. For the production of therapeutic proteins, this method may be adapted to engineer cell lines in which any desired glycosylation structure may be obtained. The invention is illustrated by production of the kringle 3 domain of **human** plasminogen and interferon-.**beta**. in engineered Pichia pastoris or Kluyveromyces lactis strains. N-glycans of secreted kringle 3 glycoproteins from Pichia pastoris strains had masses corresponding to GlcNAc1-3Man3-5GlcNAc2.

L5 ANSWER 3 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:355085 HCAPLUS
DOCUMENT NUMBER: 140:369944
TITLE: **Human** tissue-specific housekeeping genes identified by expression profiling
INVENTOR(S): Aburatani, Hiroyuki; Yamamoto, Shogo
PATENT ASSIGNEE(S): NGK Insulators, Ltd., Japan
SOURCE: PCT Int. Appl., 372 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004035785	A1	20040429	WO 2002-JP10753	20021016

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: WO 2002-JP10753 20021016
AB Housekeeping genes commonly expressed in 35 different **human** tissues, oligonucleotide probes and DNA microarrays containing them, are disclosed.
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:143277 HCAPLUS
DOCUMENT NUMBER: 140:176226
TITLE: Use of polyionic organic acids to enhance transfection efficiency and neutralize viruses
INVENTOR(S): Bennett, Michael J.; Chen, Yen-ju; Niedzinski, Edmund J.; Tseng, Hsien; Tucker, Sean
PATENT ASSIGNEE(S): Genteric, Inc., USA
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004015089	A2	20040219	WO 2003-US25419	20030812
WO 2004015089	A3	20040715		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:			
	US 2002-402811P	P	20020812
	US 2003-453999P	P	20030311
	US 2003-476145P	P	20030604

AB The present invention provides a nucleic acid transfection composition comprising a polyionic organic acid and a nucleic acid. Preferably, the polyionic organic acid is a dye. Efficient methods are also provided for administering the compns., increasing the transfection efficiency in a cell (e.g., secretory gland cell), and using the compns. as nucleic acid stabilizers to prevent in vivo and in vitro nucleic acid degradation by nucleases, thus increasing the half-life and shelf life of a nucleic acid. Addnl., polyionic organic acids may be used to neutralize viruses and to enhance effectiveness of DNA vaccines against viruses. Thus, Evans Blue and Congo Red enhanced alkaline phosphatase (SEAP) gene delivery into salivary glands resulting in high levels of secretion of SEAP into the blood. A mixture of aurintricarboxylic acid and ZnCl2 enhanced in vivo salivary gland transfection efficiency.

L5 ANSWER 5 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2003:670045 HCAPLUS
DOCUMENT NUMBER:	139:228660
TITLE:	Nuclear cataract caused by a lack of DNA degradation in the mouse eye lens
AUTHOR(S):	Nishimoto, Sogo; Kawane, Kohki; Watanabe-Fukunaga, Rie; Fukuyama, Hidehiro; Ohsawa, Yoshiyuki; Uchiyama, Yasuo; Hashida, Noriyasu; Ohguro, Nobuyuki; Tano, Yasuo; Morimoto, Takeshi; Fukuda, Yutaka; Nagata, Shigekazu
CORPORATE SOURCE:	Department of Genetics, Osaka Univ. Med. Sch., Osaka, 565-0871, Japan
SOURCE:	Nature (London, United Kingdom) (2003), 424(6952), 1071-1074 CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER:	Nature Publishing Group
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The eye lens is composed of fiber cells, which develop from the epithelial cells on the anterior surface of the lens. Differentiation into a lens fiber cell is accompanied by changes in cell shape, the expression of crystallins and the degradation of cellular organelles. The loss of organelles is believed to ensure the transparency of the lens, but the mol. mechanism behind this process is not known. Here the authors show that DLAD ('**DNase II**-like acid DNase', also called **DNase II.beta.**) is expressed in **human** and **murine** lens cells, and that mice deficient in the DLAD gene are incapable of degrading DNA during lens cell differentiation - the undigested DNA accumulates in the fiber cells. The DLAD-/- mice develop cataracts of the nucleus lentis, and their response to light on electroretinograms is severely reduced. These results indicate that DLAD

is responsible for the degradation of nuclear DNA during lens cell differentiation, and that if DNA is left undigested in the lens, it causes cataracts of the nucleus lentis, blocking the light path.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:400023 SCISEARCH
THE GENUINE ARTICLE: 676CB
TITLE: Structural requirements of **human** DNase II alpha for formation of the active enzyme: the role of the signal peptide, N-glycosylation, and disulphide bridging
AUTHOR: MacLea K S; Krieser R J; Eastman A (Reprint)
CORPORATE SOURCE: Dartmouth Coll Sch Med, Dept Pharmacol & Toxicol, 7650 Remson, Hanover, NH 03755 USA (Reprint); Dartmouth Coll Sch Med, Dept Pharmacol & Toxicol, Hanover, NH 03755 USA
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMICAL JOURNAL, (1 MAY 2003) Vol. 371, Part 3, pp. 867-876.
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND.
ISSN: 0264-6021.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 44

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB DNasc IIalpha (EC 3.1.22.1) is an endonuclease, which is active at low pH, that cleaves double-stranded DNA to short 3-phosphoryl oligonucleotides. Although its biochemistry is well understood, its structure-activity relationship has been largely unexamined. Recently, we demonstrated that active DNase IIalpha consists of one contiguous polypeptide, heavily glycosylated, and containing at least one intrachain disulphide linkage [MacLea, Krieser and Eastman (2002) Biochem. Biophys. Res. Commun. 292, 415-421]. The present paper describes further work to examine the elements of DNase IIalpha protein required for activity. Truncated forms and site-specific mutants were expressed in DNase IIalpha-null mouse cells. Results indicate that the signal-peptide leader sequence is required for correct glycosylation and that N-glycosylation is important for formation of the active enzyme. Despite this, enzymic deglycosylation of wild-type protein with peptide N-glycosidase F reveals that glycosylation is not intrinsically required for DNasc activity. DNase IIalpha contains six evolutionarily conserved cysteine residues and mutations in any one of these cysteines completely ablated enzymic activity, consistent with the importance of disulphide bridging in maintaining correct protein structure. We also demonstrate that a mutant form of DNase IIalpha that lacks the purported active-site His(295) can still bind DNA, indicating that this histidine residue is not simply involved in DNA binding, but may have a direct role in catalysis. These results provide a more complete model of the DNase IIalpha protein structure, which is important for three-dimensional structural analysis and for production of DNase IIalpha as a potential protein therapeutic for cystic fibrosis or other disorders.

L5 ANSWER 7 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:255218 SCISEARCH
THE GENUINE ARTICLE: 654ZD
TITLE: A family history of **deoxyribonuclease** - **II**: surprises from Trichinella spiralis and Burkholderia pseudomallei
AUTHOR: MacLea K S; Krieser R J; Eastman A (Reprint)
CORPORATE SOURCE: Dartmouth Coll, Sch Med, Dept Pharmacol & Toxicol, 7650 Remsen, Hanover, NH 03755 USA (Reprint); Dartmouth Coll,

Sch Med, Dept Pharmacol & Toxicol, Hanover, NH 03755 USA
COUNTRY OF AUTHOR: USA
SOURCE: GENE, (13 FEB 2003) Vol. 305, No. 1, pp. 1-12.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0378-1119.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acidic endonuclease found in lysosomes and nuclei, and it is also secreted. Though its Caenorhabditis elegans homolog, NUC-1, is required for digesting DNA of apoptotic cell corpses and dietary DNA, it is not required for viability. However, DNase IIalpha is required in mice for correct development and viability, because undigested cell corpses lead to lesions throughout the body. Recently, we showed that, in contrast to previous reports, active DNase IIalpha consists of one contiguous polypeptide. To better analyze DNase II protein structure and determine residues important for activity, extensive database searches were conducted to find distantly related family members. We report 29 new partial or complete homologs from 21 species. Four homologs with differences at the purported active site histidine residue were detected in the parasitic nematodes Trichinella spiralis and Trichinella pseudospiralis. When these mutations were reconstructed in **human** DNase IIalpha, the expressed proteins were inactive. DNase II homologs were also identified in non-metazoan species. In particular, the slime-mold Dictyostelium, the protozoan Trichomonas vaginalis, and the bacterium Burkholderia pseudomallei all contain sequences with significant similarity and identity to previously cloned DNase II family members. We report an analysis of their sequences and implications for DNase II protein structure and evolution.

L5 ANSWER 8 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:213745 HCAPLUS
DOCUMENT NUMBER: 136:227991
TITLE: Protein and cDNA sequences of **human** and mouse **deoxyribonuclease II** isoenzyme sequence homologs
INVENTOR(S): Eastman, Alan Richard; Krieser, Ronald Joe
PATENT ASSIGNEE(S): Trustees of Dartmouth College, USA
SOURCE: U.S., 8 pp., Cont.-in-part of U.S. Ser. No. 541,840.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6358723	B1	20020319	US 2000-574942	20000519
WO 2001075082	A1	20011011	WO 2001-US10635	20010402
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
US 2002028495	A1	20020307	US 2001-949434	20010907
US 6767997	B2	20040727		
PRIORITY APPLN. INFO.:			US 2000-541840	A2 20000403
			US 2000-574942	A 20000519

AB The present invention provides protein cDNA sequences of novel **DNase II** isoenzyme sequence homologs as well as vectors comprising the cDNA sequences. The invention further discloses that **human DNase II** isoenzyme gene maps on chromosome 1p22. The invention also relates to antibodies against this

protein and antisense agents targeted to a cDNA or corresponding mRNA encoding **DNase II** isoforms. In addition, methods of identifying and using modulators of **DNase II** isoform activity are described.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:219466 HCAPLUS
DOCUMENT NUMBER: 137:18119
TITLE: Gene expression profiling identifies significant differences between the molecular phenotypes of bone marrow-derived and circulating **human** CD34+ hematopoietic stem cells
AUTHOR(S): Steidl, Ulrich; Kronenwett, Ralf; Rohr, Ulrich-Peter; Fenk, Roland; Kliszewski, Slawomir; Maercker, Christian; Neubert, Peter; Aivado, Manuel; Koch, Judith; Modlich, Olga; Bojar, Hans; Gattermann, Norbert; Haas, Rainer
CORPORATE SOURCE: Department of Hematology, Oncology and Clinical Immunology, University of Dusseldorf, Dusseldorf, D-40225, Germany
SOURCE: Blood (2002), 99(6), 2037-2044
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: American Society of Hematology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CD34+ hematopoietic stem cells are used clin. to support cytotoxic therapy, and recent studies raised hope that they could even serve as a cellular source for nonhematopoietic tissue engineering. Here, we examined in 18 volunteers the gene expressions of 1185 genes in highly enriched bone marrow CD34+ (BM-CD34+) or granulocyte-colony-stimulating factor-mobilized peripheral blood CD34+ (PB-CD34+) cells by means of cDNA array technol. to identify mol. causes underlying the functional differences between circulating and sedentary hematopoietic stem and progenitor cells. In total, 65 genes were significantly differentially expressed. Greater cell cycle and DNA synthesis activity of BM-CD34+ than PB-CD34+ cells were reflected by the 2- to 5-fold higher expression of 9 genes involved in cell cycle progression, 11 genes regulating DNA synthesis, and cell cycle-initiating transcription factor E2F-1. Conversely, 9 other transcription factors, including the differentiation blocking GATA2 and N-myc, were expressed 2 to 3 times higher in PB-CD34+ cells than in BM-CD34+ cells. Expression of 5 apoptosis driving genes was also 2 to 3 times greater in PB-CD34+ cells, reflecting a higher apoptotic activity. In summary, our study provides a gene expression profile of primary **human** CD34+ hematopoietic cells of the blood and marrow. Our data molecularly confirm and explain the finding that CD34+ cells residing in the bone marrow cycle more rapidly, whereas circulating CD34+ cells consist of a higher number of quiescent stem and progenitor cells. Moreover, our data provide novel mol. insight into stem cell physiol.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2002:307432 SCISEARCH
THE GENUINE ARTICLE: 538BB
TITLE: Revised structure of the active form of **human deoxyribonuclease II** alpha
AUTHOR: MacLea K S; Krieser R J; Eastman A (Reprint)
CORPORATE SOURCE: Dartmouth Coll Sch Med, Dept Pharmacol & Toxicol, 7650 Remsen, Hanover, NH 03755 USA (Reprint); Dartmouth Coll Sch Med, Dept Pharmacol & Toxicol, Hanover, NH 03755 USA
COUNTRY OF AUTHOR: USA

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (29 MAR 2002) Vol. 292, No. 2, pp. 415-421.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.
ISSN: 0006-291X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acid endonuclease found in lysosomes, nuclei, and various secretions. **Murine** DNase IIa is required for digesting the DNA of apoptotic cells after phagocytosis and for correct development and viability. DNase IIa purified from porcine spleen was previously shown to contain three peptides, two of which were thiol crosslinked, all derived by processing of a single polypeptide. Commercial bovine protein is consistent with this structure. However, screening of 18 **human** cell lines failed to demonstrate this processing, rather a 45 kDa protein was consistently observed. Incubation of cells with the N-glycosylation inhibitor tunicamycin resulted in a 37 kDa protein, which is close to the predicted formula weight. The protein also contains at least one thiol crosslink. Similar results were obtained with overexpressed DNase IIa. These results suggest that active DNase IIa consists of one contiguous polypeptide. We suggest the previous structure reflects proteolysis during protein purification. (C) 2002 Eisevier Science (USA).

L5 ANSWER 11 OF 27 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2002-03382 BIOTECHDS
TITLE: New cDNA encoding a **deoxyribonuclease-II-beta** enzyme useful for degrading DNA present in the mucous plugs in the lungs of cystic fibrosis patients; recombinant DNA-ase-II-**beta** production and isolation useful for cystic fibrosis therapy and drug screening
AUTHOR: Eastman A R; Krieser R J
PATENT ASSIGNEE: Dartmouth-Coll.
LOCATION: Hanover, NH, USA.
PATENT INFO: WO 2001075082 11 Oct 2001
APPLICATION INFO: WO 2001-US10635 2 Apr 2001
PRIORITY INFO: US 2000-574942 19 May 2000; US 2000-541840 3 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-662972 [76]

AB A cDNA encoding a DNA-ase-II-**beta** enzyme, is new. Also claimed are: a vector comprising the claimed cDNA; an isolated and purified DNA-ase-II-**beta** enzyme; an antibody against the DNA-ase-II-**beta** enzyme; determining DNA-ase-II-**beta** levels in cells, comprising contacting the cells with the above antibody and detecting binding of the antibody; an antisense oligonucleotide targeted to a DNA or mRNA encoding the DNA-ase-II-**beta**; inhibiting expression of a DNA-ase-II-**beta** enzyme in cells, comprising administering the above antisense oligonucleotide; and digesting DNA by contacting it with the DNA-ase-II-**beta** enzyme. The DNA-ase-II-**beta** may be useful to digest DNA in the mucous plugs in lungs of cystic fibrosis patients and so reduce their viscosity (disclosed). In an example, the cDNA sequence of DNA-ase-alpha was submitted to the GenBank database and a mouse cDNA EST showing high similarity was identified, purchased and sequenced. Additional EST sequences from **human** tissues were found that had similarity to this EST but contained incomplete sequences. One sequence was found to contain 932bp of the gene referred to here as DNA-ase-II-**beta**. (11pp)

L5 ANSWER 12 OF 27 EMBASE

on STN
ACCESSION NUMBER: 2001336146 EMBASE
TITLE: Poly(amidoamine)s as potential nonviral vectors: Ability to form interpolyelectrolyte complexes and to mediate transfection in vitro.
AUTHOR: Richardson S.C.W.; Pattrick N.G.; Stella Man Y.K.; Ferruti P.; Duncan R.
CORPORATE SOURCE: R. Duncan, Centre for Polymer Therapeutics, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, United Kingdom. DUNCANR@cf.ac.uk
SOURCE: Biomacromolecules, (2001) 2/3 (1023-1028).
Refs: 35
ISSN: 1525-7797 CODEN: BOMAF6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
039 Pharmacy
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Poly(amidoamine)s (PAAs) are water-soluble polymers that display pH-dependent membrane activity. PAAs have the potential to act as a synthetic alternative to fusogenic peptides and thus promote endosomal escape. The purpose of this study was to investigate for the first time whether PAA have the ability to complex DNA, protect it from nuclease degradation and to promote transfection in vitro. PAAs ISA 1 (M(n) 6900) and ISA 23 (M(n) 10 500) and their 2-phenylethylamine containing analogues ISA 4 and ISA 22 (M(n) .apprx.8000) were studied. All PAAs retarded the electrophoretic mobility of λ Hind III DNA demonstrating interpolyelectrolyte complex (IPEC) formation and toroids of 80-150 nm in diameter (10:1 polymer excess) were visible using TEM. DNase II inhibition was observed. At a polymer:DNA ratio of 10:1, this was ISA 1(89.6 ± 6.1%), ISA 4 (92.2 ± 11.2%), ISA 22 (69.4 ± 3.7%), and ISA 23 (58.0 ± 10.0%). PAAs demonstrated the ability to mediate pSV .**beta** .-galactosidase transfection of HepG2 cells. At a vector:DNA mass ratio of 5:1, ISA 23 showed equivalent transfection ability compared with polyethylenimine and LipofectIN and was more effective than LipofectACE. These properties suggest that PAAs warrant further development as endosomolytic vectors.

L5 ANSWER 13 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:918699 HCAPLUS
DOCUMENT NUMBER: 137:91938
TITLE: Gene expression profiling of amyloid **beta** peptide-stimulated **human** post-mortem brain microglia
AUTHOR(S): Walker, Douglas G.; Lue, Lih-Fen; Beach, Thomas G.
CORPORATE SOURCE: Sun Health Research Institute, Sun City, AZ, 85351, USA
SOURCE: Neurobiology of Aging (2001), 22(6), 957-966
CODEN: NEAGDO; ISSN: 0197-4580
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Activation of microglia is a central part of the chronic inflammatory processes in Alzheimer's disease (AD). In the brains of AD patients, activated microglia are associated with amyloid **beta** (A. **beta**.) peptide plaques. A number of previous studies have shown that aggregated synthetic A.**beta**. peptide activates cultured microglia to produce a range inflammatory products. The full extent of the inflammatory response still remains to be determined In this study, gene array technol. was employed to investigate in a more extensive manner the consequences of microglial activation by A.**beta**. peptide. RNA was prepared from pooled samples of cortical **human** microglia

isolated from post-mortem cases and incubated with a low dose (2.5 μM) of A.**beta**.1-42 (or peptide solvent) for 24 h. This material was used to prepare cDNA probes, which were used to detect the differential pattern of expressed genes on a 1,176 Clontech membrane gene array. Results obtained showed that 104 genes were either upregulated or downregulated by 1.67 fold or greater. The most highly induced genes belonged to the chemokine family with interleukin-8 (IL-8) expression being increased by 11.7 fold. Interestingly, many of the highly induced genes had been identified as being responsive to activation by the transcription factor NF-κB. A number of genes were downregulated. Thymosin **beta**, prothymosin alpha and parathymosin, all belonging to the same gene family, were downregulated. To validate these semi-quant. results, the expression of intercellular adhesion mol.-1 (ICAM-1) and rhoB were measured by RT-PCR in samples of cDNA derived from A.**beta**. and control stimulated **human** cortical microglia. These results confirm the usefulness of the gene array approach for studying A.**beta**.-mediated inflammatory processes.

REFERENCE COUNT: 73 THERE ARE 73 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 14 OF 27 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2

ACCESSION NUMBER: 2001193898 EMBASE

TITLE: The cloning, genomic structure, localization, and expression of **human deoxyribonuclease II.beta**..

AUTHOR: Krieser R.J.; MacLea K.S.; Park J.P.; Eastman A.

CORPORATE SOURCE: A. Eastman, Department of Pharmacology, Dartmouth Medical School, 7650 Remsen, Hanover, NH, United States. alan.eastman@dartmouth.edu

SOURCE: Gene, (16 May 2001) 269/1-2 (205-216).
Refs: 28
ISSN: 0378-1119 CODEN: GENED6

PUBLISHER IDENT.: S 0378-1119(01)00434-6

COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
022 Human Genetics

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Acidic endonuclease activity is present in all cells in the body and much of this can be attributed to the previously cloned and ubiquitously expressed **deoxyribonuclease II** (DNase II). Database analysis revealed the existence of expressed sequence tags and genomic segments coding for a protein with considerable homology to DNase II. This report describes the cloning of this cDNA, which we term **deoxyribonuclease II.beta**. (DNase II. **beta**.) and comparison of its expression to that of the originally cloned DNase II (now termed DNase IIα). The cDNA encodes a 357 amino acid protein. This protein exhibits extensive homology to DNase IIα including an amino-terminal signal peptide and a conserved active site, and has many of the regions of identity that are conserved in homologs in other mammals as well as C. elegans and Drosophila. The gene encoding DNase II.**beta**. has identical splice sites to DNase IIα. **Human** DNase II.**beta**. is highly expressed in the salivary gland, and at low levels in trachea, lung, prostate, lymph node, and testis, whereas DNase IIα is ubiquitously expressed in all tissues. The expression pattern of **human** DNase II.**beta**. suggests that it may function primarily as a secreted enzyme. **Human** saliva was found to contain DNase IIα, but after immunodepletion, considerable acid-active endonuclease remained which we presume is DNase II.**beta**.. We have localized the gene for **human** DNase II.**beta**. to chromosome 1p22.3 adjacent (and in opposing orientation) to the **human** uricase pseudogene.

Interestingly, **murine** DNase II.**beta**. is highly expressed in the liver. Uricase is also highly expressed in mouse but not **human** liver and this may explain the difference in expression patterns between **human** and mouse DNase II.**beta**..

L5 ANSWER 15 OF 27 EMBASE
on STN DUPLICATE 3

ACCESSION NUMBER: 2001386833 EMBASE
TITLE: α,. **beta**.-poly(asparthylhydrazide)-glycidyltrimethylammonium chloride copolymers (PAHy-GTA): Novel polymers with potential for DNA delivery.
AUTHOR: Pedone E.; Cavallaro G.; Richardson S.C.W.; Duncan R.; Giammona G.
CORPORATE SOURCE: G. Giammona, Dipto. Chim./Tecnol. Farmaceutiche, Universita' degli Studi di Palermo, via Archirafi 32, 90123 Palermo, Italy. gaegiamm@unipa.it
SOURCE: Journal of Controlled Release, (9 Nov 2001) 77/1-2 (139-153).
Refs: 36
ISSN: 0168-3659 CODEN: JCREEC
PUBLISHER IDENT.: S 0168-3659(01)00459-X
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
037 Drug Literature Index
039 Pharmacy
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Hydrophilic polycations form complexes when mixed with plasmids. Following functionalisation with glycidyltrimethylammonium chloride (GTA) α,. **beta**.-poly(asparthylhydrazide) (PAHy), a water-soluble synthetic macromolecule, becomes polycationic and potentially useful for systemic gene delivery. Initially the biocompatibility of PAHy and PAHy-GTA derivatives with different degrees of positive charge substitution were studied and it was shown that PAHy-GTA was neither haemolytic nor cytotoxicity up to 1 mg/ml. After intravenous injection (125)I-labelled PAHy-GTA derivative containing 46 mol% (PAHy-GTA(b)) of trimethylammonium groups did not accumulate in the liver (4.1±0.9% of the recovered dose after 1 h) but was subjected to renal excretion (45±21% of the recovered dose was in the kidneys after 1 h). PAHy-GTA formed complexes with DNA (gel retardation) and they protected against degradation by DNase II. Finally the ability of the PAHy-GTA(b) derivative to mediate the transfection of HepG2 cells using the marker gene .**beta**.-galactosidase was studied. The optimum plasmid/polymer mass ratio was examined in comparison to LipofectACE®, Lipofectin® and polyethylenimine. .COPYRGT. 2001 Elsevier Science B.V. All rights reserved.

L5 ANSWER 16 OF 27 HCAPLUS
ACCESSION NUMBER: 2000:734130 HCAPLUS
DOCUMENT NUMBER: 134:38759
TITLE: TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix
AUTHOR(S): Yu, Wei-Hsuan; Yu, Shuan-Su C.; Meng, Qi; Brew, Keith; Woessner, J. Frederick, Jr.
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, 33101, USA
SOURCE: Journal of Biological Chemistry (2000), 275(40), 31226-31232
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Of the four known tissue inhibitors of metalloproteinases (TIMPs), TIMP-3 is distinguished by its tighter binding to the extracellular matrix. The present results show that glycosaminoglycans such as heparin, heparan sulfate, chondroitin sulfates A, B, and C, and sulfated compds. such as suramin and pentosan efficiently extract TIMP-3 from the postpartum rat uterus. Enzymic treatment by heparinase III or chondroitinase ABC also releases TIMP-3, but neither one alone gives complete release. Confocal microscopy shows co-localization of heparan sulfate and TIMP-3 in the endometrium subjacent to the lumen of the uterus. Immunostaining of TIMP-3 is lost upon digestion of tissue sections with heparinase III and chondroitinase ABC. The N-terminal domain of **human** TIMP-3 was expressed and found to bind to heparin with affinity similar to that of full-length mouse TIMP-3. The A and B **.beta.**-strands of the N-terminal domain of TIMP-3 contain two potential heparin-binding sequences rich in lysine and arginine; these strands should form a double track on the outer surface of TIMP-3. Synthetic peptides corresponding to segments of these two strands compete for heparin in the **DNase II** binding assay. TIMP-3 binding may be important for the cellular regulation of activity of the matrix metalloproteinases.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 17 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:801315 HCAPLUS
DOCUMENT NUMBER: 134:98363
TITLE: Towards a **human** repertoire of monocytic lysosomal proteins
AUTHOR(S): Journet, Agnes; Chapel, Agnes; Kieffer, Sylvie; Louwagie, Mathilde; Luche, Sylvie; Garin, Jerome
CORPORATE SOURCE: Laboratoire de Chimie des Proteines, CEA-Grenoble, Grenoble, F-38054, Fr.
SOURCE: Electrophoresis (2000), 21(16), 3411-3419
CODEN: ELCTDN; ISSN: 0173-0835
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lysosomal compartment of **human** monocytic cells has never been investigated by a proteomic approach. By a combination of one-dimensional (1-D) and two-dimensional (2-D) gel electrophoresis, protein identification by N-terminal sequencing, matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS) peptide mass fingerprinting and tandem mass spectrometry (MS/MS) peptide sequence anal., we initiated an exhaustive study of the **human** lysosomal proteome, which aims at establishing a 2-D reference map of **human** soluble lysosomal proteins. **Human** monocytic U937 cells were induced to secrete lysosomal soluble hydrolases by addition of NH4Cl in the culture medium. Since lysosomal soluble proteins are characterized by the presence of mannose-6-phosphate, they were purified on an affinity support bearing mannose-6-phosphate receptor. Anal. of the purified fraction led to the preliminary identification of fifteen proteins, among which twelve are well-known lysosomal hydrolases, one is assumed to be lysosomal on the basis of sequence homol. to cysteine proteinases of the papain family, and two (leukocystatin and the **human** cellular repressor of E1A-stimulated genes) are described here for the first time as mannose-6-phosphate-containing proteins.
REFERENCE COUNT: 69 THERE ARE 69 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 18 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 4
ACCESSION NUMBER: 1998:706859 SCISEARCH
THE GENUINE ARTICLE: 118MG

TITLE: Enhanced reporter gene expression in cells transfected in the presence of DMI-2, an acid nuclease inhibitor
AUTHOR: Ross G F (Reprint); Bruno M D; Uyeda M; Suzuki K; Nagao K; Whitsett J A; Korfhagen T R
CORPORATE SOURCE: CHILDRENS HOSP, MED CTR, DIV PULM BIOL, 3333 BURNET AVE, CINCINNATI, OH 45229 (Reprint); KUMAMOTO UNIV, DIV PHARMACEUT SCI, KUMAMOTO, JAPAN
COUNTRY OF AUTHOR: USA; JAPAN
SOURCE: GENE THERAPY, (SEP 1998) Vol. 5, No. 9, pp. 1244-1250. Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND. ISSN: 0969-7128.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 26

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cellular nuclease activity is a potential barrier to the successful delivery of foreign genes to mammalian cells We tested the hypothesis that transfection in the presence of a specific DNase inhibitor can enhance the expression of foreign gene products. We have used DMI-2, a polyketide metabolite of Streptomyces sp. strain 560 to enhance the expression of bacterial chloramphenicol acetyltransferase (CAT) in the **human** lung adenocarcinoma cell line H441. DMI-2 has been shown previously to inhibit porcine DNase II, an acid pH nuclease contained in the endosomal/lysosomal compartment. Transfection of H441 cells in the presence of 0.1-1 mu g/ml DMI-2 caused: (1) 10-fold enhancement of CAT activity when the bacterial plasmid was complexed with either surfactant protein A-poly-lysine or transferrin-poly-lysine; (2) 1.5- to two-fold enhancement of CAT activity in cells exposed to lipofectin-DNA complexes; (3) no effect on transfection via calcium phosphate co-precipitation. DMI-2 alone showed no inherent transfection activity. In experiments using SP-A-poly-lysine and plasmid containing the **beta**-galactosidase reporter gene, DMI-2 increased the number of transfected cells. Methanolysis products of DMI-2 did not inhibit DNase II and did not enhance transfection efficiency, Taken together, the data support the hypothesis that nuclease action is a significant barrier to expression of-foreign genes and inhibition of specific nucleases may facilitate transfection.

L5 ANSWER 19 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1998:144482 SCISEARCH
THE GENUINE ARTICLE: YW407
TITLE: Properties of the endonuclease secreted by **human** B lymphoblastic IM9 cells
AUTHOR: Kwon H J (Reprint); Kim D S
CORPORATE SOURCE: YONSEI UNIV, COLL SCI, DEPT BIOCHEM, SEOUL 120749, SOUTH KOREA; YONSEI UNIV, BIOPROD RES CTR, SEOUL 120749, SOUTH KOREA
COUNTRY OF AUTHOR: SOUTH KOREA
SOURCE: JOURNAL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, (31 JAN 1998) Vol. 31, No. 1, pp. 106-110. Publisher: BIOCHEMICAL SOC REPUBLIC KOREA, KOREA SCI TECHNOLOGY CENTER, RM 801, 635-4 YEOGSAM-DONG, KANGNAM-GU, SEOUL 135-753, SOUTH KOREA. ISSN: 1225-8687.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have employed a DNA-native-polyacrylamide gel electrophoresis (DNA-native-PAGE) assay system to characterize the enzyme activity of the endonuclease secreted by **human** B lymphoblastic IM9 cells.

Experimental results clearly demonstrated that the endonuclease activity of IM9 cell culture medium is distinct from that of DNase I in the DNA-native-PAGE assay system, Immunoprecipitation analysis using anti-DNase I antibody showed that the secreted endonuclease is not recognized by the antibody, The secreted endonuclease was isolated from the cell culture medium by native-PAGE elution technique, and the enzyme activity was estimated using supercoiled plasmid DNA as a substrate. The pH optimum required for the catalytic activity was determined to be in the range of pH 6.6-7.4. No significant difference in the endonuclease secretion was observed by stimulation of the IM9 cells with interferon-gamma or interleukin-1 **beta**.

L5 ANSWER 20 OF 27 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 97:108926 SCISEARCH
THE GENUINE ARTICLE: WE588
TITLE: Zinc inhibits apoptosis upstream of ICE/CED-9 proteases rather than at the level of an endonuclease
AUTHOR: Wolf C M; Morana S J; Eastman A (Reprint)
CORPORATE SOURCE: DARTMOUTH COLL, SCH MED, DEPT PHARMACOL & TOXICOL, HANOVER, NH 03755 (Reprint); DARTMOUTH COLL, SCH MED, DEPT PHARMACOL & TOXICOL, HANOVER, NH 03755
COUNTRY OF AUTHOR: USA
SOURCE: CELL DEATH AND DIFFERENTIATION, (FEB 1997) Vol. 4, No. 2, pp. 125-129.
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS.
ISSN: 1350-9047.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Apoptosis is commonly associated with DNA digestion, but it remains controversial as to which endonuclease is involved. The ability of zinc to inhibit DNA digestion in intact cells, and inhibit a Ca2+/Mg2+-dependent endonuclease in cell lysates, has been used frequently to suggest this is the endonuclease involved. However, zinc has many other effects on cells, and here it is shown that zinc also prevents many upstream events in apoptosis. These studies were performed in **human** ML-1 cells following incubation with etoposide. During apoptosis, these cells undergo intracellular acidification, increased accumulation of Hoechst 33342, DNA digestion and chromatin condensation. Zinc inhibited all of these events. An upstream event in apoptosis is activation of ICE/CED-3 proteases which is commonly observed as proteolysis of a substrate protein, poly(ADP-ribose) polymerase (PARP). The ICE/CED-3 proteases are themselves activated by proteolysis, and this was detected here by cleavage of one family member CPP32. Zinc prevented cleavage of both CPP32 and PARP. We recently demonstrated that dephosphorylation of the retinoblastoma susceptibility protein Rb was a marker of an event even further upstream in apoptosis; zinc was also found to inhibit Rb dephosphorylation. Therefore, zinc must protect cells at a very early step in the apoptotic pathway, and not as a direct inhibitor of an endonuclease.

L5 ANSWER 21 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:187374 HCAPLUS
DOCUMENT NUMBER: 118:187374
TITLE: Method using two-component additive for stabilization of biomaterials during lyophilization
INVENTOR(S): Carpenter, John F.
PATENT ASSIGNEE(S): Cryolife, Inc., USA
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9300807	A1	19930121	WO 1992-US5643	19920702
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO				
AU 9223096	A1	19930211	AU 1992-23096	19920702
PRIORITY APPLN. INFO.:			US 1991-725593	19910703
			WO 1992-US5643	19920702

AB A method for stabilizing biomaterials during lyophilization uses a two-component additive. The 1st component (PEG, dextran, ficoll, etc.) serves as a cryoprotectant, and the 2nd component (e.g. a sugar polyhydroxy alc., amino acid) protects the biomaterial (e.g. a protein) during drying. In freeze-drying lactate dehydrogenase M isoenzyme with PEG and a second component (trehalose, lactose, glucose, glycine, or mannitol), the results supported synergistic stabilization of the protein during freeze-drying.

L5 ANSWER 22 OF 27 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 83231243 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6190491
TITLE: Cytochemical comparison of immunologically characterized **human** leukaemia/lymphoma cell lines representing different levels of maturation.
AUTHOR: Srivastava B I; Rossowski W; Minowada J
CONTRACT NUMBER: CA-14413 (NCI)
CA-17140 (NCI)
SOURCE: British journal of cancer, (1983 Jun) 47 (6) 771-9.
Journal code: 0370635. ISSN: 0007-0920.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198308
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19830811

AB Forty-seven **human** leukaemia/lymphoma cell lines belonging to myelocytic, monocytic, non-T/non-B, T-, and B-lineage and representing different levels of maturation as well as fresh cells from normal and leukaemic subjects were examined for immunological markers and cytochemically for acid phosphatase, alkaline phosphatase, alpha-naphthyl acetate esterase (pH 5.8 and 8.0), alpha-naphthyl butyrate esterase (pH 5.8 and 8.0), non-specific esterase, chloroacetate esterase, chymotrypsin-like protease, **deoxyribonuclease II**, **beta**-glucuronidase, sudan black, and periodic acid Schiff's staining. Strong sudan black, nonspecific esterase, and chloroacetate esterase reaction was obtained only for myelocytic and monocytic cell lines with the reaction intensity increasing progressively in more mature cells. Focal acid phosphatase reaction like T-ALL was found in all T-ALL cell lines, whereas myeloid/monocytoid lines had semicircular distribution and B-cell lines cytoplasmic distribution of activity. Acid phosphatase activity appeared to decline with maturation along both myeloid and T-cell lineage. High activity of alpha-naphthyl acetate esterase and alpha-naphthyl butyrate esterase both at pH 5.8 and 8.0 and of **beta**-glucuronidase was found in myeloid/monocytoid lines although both B- and T-cell lines in contrast to peripheral blood B-cells also had significant esterase activity. alpha-Naphthyl butyrate esterase activity declined with increasing cell maturation along myeloid lineage. Except for weak activity in two B-cell lines alkaline phosphatase was not detected in any cell lines. Monocyte esterase activity was inhibited by

sodium fluoride whereas acid phosphatase, only from hairy cell leukaemia line, was resistant to L-tartarate. Although periodic acid Schiff's staining could not distinguish myeloid, T-, B-, or non-T/non-B cell lines it gave characteristic reaction (large number of coarse granules against a clear background forming a ring around the nucleus) with erythroblastic leukaemia cell line and along myeloid series its intensity increased in more mature cells. **Deoxyribonuclease II** and chymotrypsin-like protease staining were not discriminatory. The results of this study show that cytochemical staining characteristics of various leukaemia/lymphoma cell lines are comparable to those of corresponding cells from patients and that the intensity and pattern of expression of these activities are related to cell type and degree of cell maturation. These studies give further credence to the use of these cell lines in cell differentiation, differential drug cytotoxicity, and many other studies.

L5 ANSWER 23 OF 27 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 83178349 EMBASE
DOCUMENT NUMBER: 1983178349
TITLE: Cytochemical comparison of immunologically characterized **human** leukaemia/lymphoma cell lines representing different levels of maturation.
AUTHOR: Sahai Srivastava B.I.; Rossowski W.; Minowada J.
CORPORATE SOURCE: Dep. Exp. Ther., Roswell Park Meml. Inst., Buffalo, NY 14263, United States
SOURCE: British Journal of Cancer, (1983) 47/6 (771-779). CODEN: BJCAAI
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal
FILE SEGMENT: 016 Cancer
025 Hematology
026 Immunology, Serology and Transplantation
005 General Pathology and Pathological Anatomy
LANGUAGE: English

AB Forty-seven **human** leukaemia/lymphoma cell lines belonging to myelocytic, monocytic, non-T/non-B, T-, and B-lineage and representing different levels of maturation as well as fresh cells from normal and leukaemic subjects were examined for immunological markers and cytochemically for acid phosphatase, alkaline phosphatase, α-naphthyl acetate esterase (pH 5.8 and 8.0), α-naphthyl butyrate esterase (pH 5.8 and 8.0), non-specific esterase, chloroacetate esterase, chymotrypsin-like protease, **deoxyribonuclease II**, .**beta**.-glucuronidase, sudan black, and periodic acid Schiff's staining. Strong sudan black, nonspecific esterase, and chloroacetate esterase reaction was obtained only for myelocytic and monocytic cell lines with the reaction intensity increasing progressively in more mature cells. Focal acid phosphatase reaction like T-ALL was found in all T-ALL cell lines, whereas myeloid/monocytoid lines had semicircular distribution and B-cell lines cytoplasmic distribution of activity. Acid phosphatase activity appeared to decline with maturation along myeloid and T-cell lineage. High activity of α-naphthyl acetate esterase and α-naphthyl butyrate esterase both at pH 5.8 and 8.0 and of . **beta**.-glucuronidase was found in myeloid/monocytoid lines although both B- and T-cell lines in contrast to peripheral blood B-cells also had significant esterase activity. α-Naphthyl butyrate esterase activity declined with increasing cell maturation along myeloid lineage. Except for weak activity in two B-cell lines alkaline phosphatase was not detected in any cell lines. Monocyte esterase activity was inhibited by sodium fluoride whereas acid phosphatase, only from hairy cell leukaemia line, was resistant to L-tartarate. Although periodic acid Schiff's staining could not distinguish myeloid, T-, B-, or non-T/non-B cell lines it gave characteristic reaction (large number of coarse granules against a clear background forming a ring around the nucleus) with erythroblastic leukaemia cell line and along myeloid series its intensity increased in

more mature cells. **Deoxyribonuclease II** and chymotrypsin-like protease staining were not discriminatory. The results of this study show that cytochemical staining characteristics of various leukaemia/lymphoma cell lines are comparable to those of corresponding cells from patients and that the intensity and pattern of expression of these activities are related to cell type and degree of cell maturation. These studies give further credence to the use of these cell lines in cell differentiation, differential drug cytotoxicity, and many other studies.

L5 ANSWER 24 OF 27 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 83:37947 LIFESCI
TITLE: Cytochemical comparison of immunologically characterized **human** leukaemia/lymphoma cell lines representing different levels of maturation.
AUTHOR: Sahai Srivastava, B.I.; Rossowski, W.; Minowada, J.
CORPORATE SOURCE: Dep. Exp. Ther. and Grace Cancer Drug Cent., 666 Elm St., Buffalo, NY 14263, USA
SOURCE: BR. J. CANCER., (1983) vol. 47, no. 6, pp. 771-779.
DOCUMENT TYPE: Journal
FILE SEGMENT: F
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Forty-seven **human** leukaemia/lymphoma cell lines belonging to myelocytic, monocytic, non-T/non-B, T-, and B-lineage and representing different levels of maturation as well as fresh cells from normal and leukaemic subjects were examined for immunological markers and cytochemically for acid phosphatase, alkaline phosphatase, alpha -naphthyl acetate esterase (pH 5.8 and 8.0), alpha -naphthyl butyrate esterase (pH 5.8 and 8.0), non-specific esterase, chloroacetate esterase, chymotrypsin-like protease, **deoxyribonuclease II**, **beta** -glucoronidase, sudan black, and periodic acid Schiff's staining. The result of this study show that cytochemical staining characteristics of various leukaemia/lymphoma cell lines are comparable to those of corresponding cells from patients and that the intensity and pattern of expression of these activities are related to cell type and degree of cell maturation. These studies give further credence to the use of these cell lines in cell differentiation, differential drug cytotoxicity, and many other studies.

L5 ANSWER 25 OF 27 MEDLINE on STN
ACCESSION NUMBER: 83257382 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6307389
TITLE: Implications of a 5'-nucleotidase inhibitor in **human** leukemic cells for cellular aging and cancer.
AUTHOR: Sun A S; Holland J F; Lin K; Ohnuma T
SOURCE: Biochimica et biophysica acta, (1983 Jul 14) 762 (4) 577-84.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198309
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19980206
Entered Medline: 19830909
AB 5'-Nucleotidase activity of normal **human** embryonic lung fibroblasts (IMR-90) was found to be inhibited by the homogenates of seven different cell lines originated from patients with different kinds of leukemia and of fresh lymphocytes from a patient with Sezary syndrome (circulating T-cell lymphoma). About 97% of the inhibiting activity was found in the soluble fraction of RPMI 8402 cells, a cell line originated from the lymphocytes of a patient with acute lymphocytic leukemia. This inhibiting activity was not destroyed by dialysis, heating at 56 degrees C

for 30 min, nor digestion with RNAase or DNAase. About 85% of the inhibiting activity was destroyed by digestion with papain at 37 degrees C for 1 h and it was destroyed completely by heating at 100 degrees C for 30 min. When the heated (56 degrees C for 30 min) soluble fraction of RPMI 8402 cells was mixed with the homogenate of IMR-90 cells, it had no effect on the activities of alkaline, neutral or acid phosphatases, nor of N-acetyl-**beta**-D-glucosaminidase or cytochrome c oxidase of IMR-90 cells. Preincubating the mixed samples for 1, 20 and 45 min, respectively, before adding the substrate, the heated soluble fraction of RPMI 8402 cells did not increase the percentage of inhibition for 5'-nucleotidase of the homogenate of IMR-90 cells. No inhibition of other enzyme activities was observed under similar conditions. These data suggest that the inhibiting activity is due to a protein(s) that is not a protease. The inhibiting activity was found in a single peak after the soluble fraction was fractionated by Sephadex G-100 chromatography and sedimentation centrifugation. The molecular weight of the inhibitor was found to be approx. 35,000 by comparing its retention volume and sedimentation rate with those of proteins of known molecular weight. The present study suggest that the previously reported undetectability of 5'-nucleotidase in permanent cell lines could be due to the presence of a protein inhibitor for 5'-nucleotidase in these **human** leukemic cell lines. It also supports the hypothesis that the increased 5'-nucleotidase activity in normal senescent cells in vitro may be a control in cellular aging that is missing from leukemic cells in vitro.

L5 ANSWER 26 OF 27 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 6

ACCESSION NUMBER: 1978:175412 BIOSIS
DOCUMENT NUMBER: PREV197865062412; BA65:62412
TITLE: QUANTITATIVE ESTIMATION OF ENZYMES IN **HUMAN** PERIPHERAL BLOOD LYMPHOCYTES PART 1 ENZYME ACTIVITIES IN LYMPHOCYTES OF HEALTHY INDIVIDUALS.
AUTHOR(S): BOGAJEWSKI J [Reprint author]; BOGAJEWSKA G; MACKIEWICZ S
CORPORATE SOURCE: ZAKL IMMUNOL, AKAD MED, SZKOLNA 8/12, POZNAN, POL
SOURCE: Immunologia Polska, (1976) Vol. 1, No. 4, pp. 285-294. CODEN: IMPODM. ISSN: 0324-8534.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: POLISH

AB Activities of the following enzymes were quantitated in peripheral blood lymphocytes of healthy individuals: lactate, glucose-6-phosphate succinate and reduced NAD dehydrogenases, alanine and aspartate dehydrogenases, carboxyloesterase, cholinesterase, acid phosphatase, **deoxyribonuclease II** and .**beta**.-glururonidase.

L5 ANSWER 27 OF 27 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1964:4249 HCAPLUS
DOCUMENT NUMBER: 60:4249
ORIGINAL REFERENCE NO.: 60:788a-c
TITLE: Autoradiographic studies of the effects of antibiotics, amino acid analogs, and nucleases on the synthesis of deoxyribonucleic acid (DNA) in cultured mammalian cells
AUTHOR(S): Shah, Vinod C.
CORPORATE SOURCE: Columbia Univ.
SOURCE: Cancer Research (1963), 23, 1137-47 CODEN: CNREA8; ISSN: 0008-5472
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB Processes suspeered of involvement in the control of DNA replication were investigated. Cultured cells of Chinese hamster and **human** cancer (HeLa) were used. Puromycin, chloramphenicol, pfluorophenylalanine, and methylated tryptophan analogs inhibited the synthesis of protein. They also affected the synthesis of DNA by changing

the number of cells able to incorporate thymidine-3H as well as by reducing the rate of thymidine incorporation in the replicating cells. The relation of histone and DNA synthesis was studied cytochem., and data obtained indicate that histones do not seem to play any direct role in DNA replication. Actinomycin D inhibited intracellular ribonueleic acid (RNA) synthesis but did not affect DNA synthesis. This suggests that there is no necessary coupling of RNA and DNA syntheses. The nucleases (DNase I, **DNase II**, and RNase), within a short time after contact with the cells in culture medium, caused an increase in the number of cells able to incorporate thymidine. After 3 hrs. of treatment, both **DNase II** and RNase resulted in decreases in DNA synthesis. Results are discussed with regard to the hypothesis that RNA may act as repressor for DNA synthesis and the repressor activity is regulated by amino acids or proteins.

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=> d his

     (FILE 'HOME' ENTERED AT 09:06:25 ON 04 NOV 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004
L1          1382 S "DEOXYRIBONUCLEASE II"
L2           377 S L1 AND (HUMAN OR MURINE)
L3       3858984 S BETA
L4            36 S L2 AND L3
L5            27 DUP REM L4 (9 DUPLICATES REMOVED)

=> s digest?
L6      1714908 DIGEST?

=> s l1 and l6
L7           348 L1 AND L6

=> s l7 and (human and murine)
<------User Break------>

SEARCH ENDED BY USER
SEARCH ENDED BY USER

=> s l7 and (human or murine)
   4 FILES SEARCHED...
L8            76 L7 AND (HUMAN OR MURINE)

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            41 DUP REM L8 (35 DUPLICATES REMOVED)

=> d 1-41 ibib ab

L9   ANSWER 1 OF 41  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2004:681680  HCAPLUS
DOCUMENT NUMBER:          141:200162
TITLE:                    Mitochondrial malate dehydrogenase DNA fragmentation
                          activator fragment and related conjugated proteins and
                          antibodies for cancer therapy
INVENTOR(S):              Wright, Susan C.; Larrick, James W.; Nock, Steffen R.;
                          Wilson, David S.
PATENT ASSIGNEE(S):       Palo Alto Institute of Molecular Medicine, USA
SOURCE:                   PCT Int. Appl., 225 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
```

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004070012	A2	20040819	WO 2004-US2974	20040202
W: AE, AE, AG, AL, AL, AM, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG, BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR, CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES, ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN, IS, JP, JP, KE, KE, KG, KG, KP, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2004191843	A1	20040930	US 2004-770668	20040202
PRIORITY APPLN. INFO.:			US 2003-444191P	P 20030203
			US 2003-460855P	P 20030408

AB The invention provides compns. comprising amino acid sequences that have cell killing activity, nucleic acid sequences encoding them, antibodies that specifically bind with them, and methods of using these compns. for increasing and/or reducing cell death, detecting cell death, diagnosing diseases associated with altered cell death, and methods for identifying test agents that alter cell death. More particularly, the invention provides an activator of DNA fragmentation (ADF), a C-terminal fragment of mitochondrial MDH (malate dehydrogenase), which can induce DNA fragmentation by activating nuclease endogenous to normal nuclei. The invention also provides a conjugate comprising a cell death-inducing mol. (such as ADF) and a cell mol.-recognizing compound, and use of said conjugate in killing cancer cells. Specifically, the invention relates that conjugate can be composed of said ADF and/or other mitochondrial/non-mitochondrial cell death-inducing proteins (such as Htra/Omi, apoptosis inducing factor, Smac/DIABLO, EndoG, Nix, Nip3, CIDE-B, gelsolin, Bcl-2, Bax, Bad, Bid, caspase-activated DNase, DNase I or **DNase II**), and that cell mol.-recognizing compds.
can include antibodies or growth factors. In particular embodiments, recombinant ADF proteins, ADF-Ant (antennapedia) and rADF-bFGF, are shown to be cytotoxic to a variety to tumor cell types, and even drug-resistant cancer cell lines.

L9 ANSWER 2 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:510285 HCAPLUS
DOCUMENT NUMBER: 141:52348
TITLE: Compounds and methods for detection of carcinomas and their precursor lesions by analyzing carcinomas marker molecule DNase expression
INVENTOR(S): Coy, Johannes
PATENT ASSIGNEE(S): MTM Laboratories Ag, Germany
SOURCE: Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1431762	A1	20040623	EP 2002-102814	20021218
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
WO 2004055514	A1	20040701	WO 2003-EP51028	20031216
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2002-102814 A 20021218

AB The present invention relates to compds. and methods for detection and treatment of carcinomas and their precursor lesions. The invention provides DNase nucleic acids and polypeptides useful for the detection and treatment of carcinomas and their precursor lesions. Specifically, this includes **human** (a) DNase 1-like 1 (DNase X) (NM_006730), (2) DNase 1-like 3 (also called DNase gamma) (AF047354), (c) DNase I (AJ298844), (d) **DNase II** (AB004574), (e) DNase 1-like 2 (AK098028), (f) caspase activated DNase (AB013918), (g) DNase KIAA0218 (D86972), (h) DNase 1-like DNase (AF274571), and (i) DFF-45 (AF087573). The invention is more specifically related to a method for detection of carcinomas and their precursor lesions comprising the detection of the level and/or the subcellular localization of one or more DNase mols. in biol. samples. Furthermore the present invention provides methods for early diagnosis, prognosis and monitoring of the disease course of carcinomas and their precursor lesions as well as for the treatment of said lesions.

L9 ANSWER 3 OF 41 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2004029194 MEDLINE

DOCUMENT NUMBER: PubMed ID: 14727918

TITLE: Formation and mass spectrometric analysis of DNA and nucleoside adducts by S-(1-acetoxymethyl)glutathione and by glutathione S-transferase-mediated activation of dihalomethanes.

AUTHOR: Marsch Glenn A; Botta Sisir; Martin Martha V; McCormick W Andrew; Guengerich F Peter

CORPORATE SOURCE: Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.

CONTRACT NUMBER: P30 ES00267 (NIEHS)
R01 ES10546 (NIEHS)

SOURCE: Chemical research in toxicology, (2004 Jan) 17 (1) 45-54. Journal code: 8807448. ISSN: 0893-228X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200409

ENTRY DATE: Entered STN: 20040121
Last Updated on STN: 20040921
Entered Medline: 20040920

AB The dihalomethane CH(2)Cl(2) is an industrial solvent of potential concern to **humans** because of its potential genotoxicity and carcinogenicity. To characterize DNA damage by dihalomethanes, a rapid DNA **digestion** under acidic conditions was developed to identify alkali labile DNA-dihalomethane nucleoside adducts using HPLC-electrospray mass spectrometry. DNA **digestion** worked best using pH 5.0 sodium acetate buffer, a 30 min incubation with DNase II and phosphodiesterase II, and a 2 h acid phosphatase **digest**. DNA was modified with S-(1-acetoxymethyl)glutathione (GSCH(2)OAc), a reagent modeling activated dihalomethanes. Adducts to G, A, and T were detected at high ratios of GSCH(2)OAc/DNA following **digestion** of the DNA with the procedure used here. The relative efficacy of adduct formation

was G > T > A >> C. The four DNA nucleosides were also reacted with the dihalomethanes CH(2)Cl(2) and CH(2)Br(2) in the presence of glutathione (GSH) and GSH S-transferases from bacteria (DM11), rat (GST 5-5), and **human** (GST T1-1) under conditions that produce mutations in bacteria. All enzymes formed adducts to all four nucleosides, with dGuo being the most readily modified nucleoside. Thus, the pattern paralleled the results obtained with the model compounds GSCH(2)OAc and DNA. CH(2)Cl(2) and CH(2)Br(2) yielded similar amounts of adducts under these conditions. The relative efficiency of adduct formation by GSH transferases was rat 5-5 > **human** T1-1 > bacterial DM11, showing that **human** GSH transferase T1-1 can form dihalomethane adducts under the conditions used. Although the lability of DNA adducts has precluded more sophisticated experiments and in vivo studies have not yet been possible, the work collectively demonstrates the ability of several GSH transferases to generate DNA adducts from dihalomethanes, with G being the preferred site of adduction in both this and the GSCH(2)OAc model system.

L9 ANSWER 4 OF 41 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003082972 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12594037
TITLE: A family history of **deoxyribonuclease II** : surprises from Trichinella spiralis and Burkholderia pseudomallei.
AUTHOR: MacLea Kyle S; Krieser Ronald J; Eastman Alan
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755, USA.
CONTRACT NUMBER: CA 23108 (NCI)
CA 50224 (NCI)
SOURCE: Gene, (2003 Feb 13) 305 (1) 1-12.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 20030221
Last Updated on STN: 20030426
Entered Medline: 20030425

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acidic endonuclease found in lysosomes and nuclei, and it is also secreted. Though its Caenorhabditis elegans homolog, NUC-1, is required for **digesting** DNA of apoptotic cell corpses and dietary DNA, it is not required for viability. However, DNase IIalpha is required in mice for correct development and viability, because undigested cell corpses lead to lesions throughout the body. Recently, we showed that, in contrast to previous reports, active DNase IIalpha consists of one contiguous polypeptide. To better analyze DNase II protein structure and determine residues important for activity, extensive database searches were conducted to find distantly related family members. We report 29 new partial or complete homologs from 21 species. Four homologs with differences at the purported active site histidine residue were detected in the parasitic nematodes Trichinella spiralis and Trichinella pseudospiralis. When these mutations were reconstructed in **human** DNase IIalpha, the expressed proteins were inactive. DNase II homologs were also identified in non-metazoan species. In particular, the slime-mold Dictyostelium, the protozoan Trichomonas vaginalis, and the bacterium Burkholderia pseudomallei all contain sequences with significant similarity and identity to previously cloned DNase II family members. We report an analysis of their sequences and implications for DNase II protein structure and evolution.

L9 ANSWER 5 OF 41 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002174344 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11906178
TITLE: Revised structure of the active form of **human** deoxyribonuclease IIalpha.
AUTHOR: MacLea Kyle S; Krieser Ronald J; Eastman Alan
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Biochemical and biophysical research communications, (2002 Mar 29) 292 (2) 415-21.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020322
Last Updated on STN: 20020507
Entered Medline: 20020506

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acid endonuclease found in lysosomes, nuclei, and various secretions. **Murine** DNase IIalpha is required for **digesting** the DNA of apoptotic cells after phagocytosis and for correct development and viability. DNase IIalpha purified from porcine spleen was previously shown to contain three peptides, two of which were thiol crosslinked, all derived by processing of a single polypeptide. Commercial bovine protein is consistent with this structure. However, screening of 18 **human** cell lines failed to demonstrate this processing, rather a 45 kDa protein was consistently observed. Incubation of cells with the N-glycosylation inhibitor tunicamycin resulted in a 37 kDa protein, which is close to the predicted formula weight. The protein also contains at least one thiol crosslink. Similar results were obtained with overexpressed DNase IIalpha. These results suggest that active DNase IIalpha consists of one contiguous polypeptide. We suggest the previous structure reflects proteolysis during protein purification.

L9 ANSWER 6 OF 41 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:379757 BIOSIS
DOCUMENT NUMBER: PREV200200379757
TITLE: Production and characterization of **murine** monoclonal anti-**human** DNase II antibodies, and their use for immunoaffinity purification of DNase II from **human** liver and urine.
AUTHOR(S): Nakajima, Tamiko; Yasuda, Toshihiro; Takeshita, Haruo; Mori, Shinjiro; Mogi, Kouichi; Kaneko, Yasushi; Nakazato, Emiko; Kishi, Koichiro [Reprint author]
CORPORATE SOURCE: Department of Legal Medicine, Gunma University School of Medicine, Maebashi, Gunma, 371-8511, Japan
kkoichi@med.gunma-u.ac.jp
SOURCE: Biochimica et Biophysica Acta, (15 April, 2002) Vol. 1570, No. 3, pp. 160-164. print.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Jul 2002
Last Updated on STN: 10 Jul 2002

AB Four **murine** monoclonal anti-**human** **deoxyribonuclease II** (DNase II) antibodies were obtained from BALB/c mice immunized with **human** DNase II purified from **human** liver. Both single radial enzyme diffusion (SRED) and DNA-cast polyacrylamide gel electrophoresis (DNA-cast PAGE) were very useful for obtaining the DNase II-specific antibodies. All of the

antibodies showed specific inhibition of **human** DNase II enzyme activity and specific immunostaining of the 32-kDa enzyme band, which is one of the three non-identical subunits of **human** DNase II molecule separated by sodium dodecyl sulfate (SDS)-PAGE followed by blotting on a transfer membrane. A formyl-cellulofine resin conjugated with each antibody specifically adsorbed and efficiently desorbed the active DNase II enzyme. Insertion of the immunoaffinity step in our purification procedure made the purification of **human** DNase II easier, faster and more effective than the conventional procedure.

L9 ANSWER 7 OF 41 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-03382 BIOTECHDS
TITLE: New cDNA encoding a **deoxyribonuclease-II** -beta enzyme useful for degrading DNA present in the mucous plugs in the lungs of cystic fibrosis patients; recombinant DNA-ase-II-beta production and isolation useful for cystic fibrosis therapy and drug screening
AUTHOR: Eastman A R; Krieser R J
PATENT ASSIGNEE: Dartmouth-Coll.
LOCATION: Hanover, NH, USA.
PATENT INFO: WO 2001075082 11 Oct 2001
APPLICATION INFO: WO 2001-US10635 2 Apr 2001
PRIORITY INFO: US 2000-574942 19 May 2000; US 2000-541840 3 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-662972 [76]
AB A cDNA encoding a DNA-ase-II-beta enzyme, is new. Also claimed are: a vector comprising the claimed cDNA; an isolated and purified DNA-ase-II-beta enzyme; an antibody against the DNA-ase-II-beta enzyme; determining DNA-ase-II-beta levels in cells, comprising contacting the cells with the above antibody and detecting binding of the antibody; an antisense oligonucleotide targeted to a DNA or mRNA encoding the DNA-ase-II-beta; inhibiting expression of a DNA-ase-II-beta enzyme in cells, comprising administering the above antisense oligonucleotide; and **digesting** DNA by contacting it with the DNA-ase-II-beta enzyme. The DNA-ase-II-beta may be useful to **digest** DNA in the mucous plugs in lungs of cystic fibrosis patients and so reduce their viscosity (disclosed). In an example, the cDNA sequence of DNA-ase-alpha was submitted to the GenBank database and a mouse cDNA EST showing high similarity was identified, purchased and sequenced. Additional EST sequences from **human** tissues were found that had similarity to this EST but contained incomplete sequences. One sequence was found to contain 932bp of the gene referred to here as DNA-ase-II-beta. (11pp)

L9 ANSWER 8 OF 41 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:245207 BIOSIS
DOCUMENT NUMBER: PREV200100245207
TITLE: Structure and processing of **human deoxyribonuclease II.**
AUTHOR(S): MacLea, Kyle S. [Reprint author]; Krieser, Ronald J. [Reprint author]; Eastman, Alan [Reprint author]
CORPORATE SOURCE: Pharmacology/Toxicology, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A202. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 2001

Last Updated on STN: 19 Feb 2002

AB **Deoxyribonuclease II** (DNase II) is an endonuclease active at acidic pH that is found in lysosomes and in various secretions. It has recently been identified as a homolog of the Caenorhabditis elegans NUC-1 protein that is required for **digesting** the DNA of apoptotic cells and ingested bacteria. The nonhomologous **human** DNase I protein is used clinically to **digest** the viscous sputum of cystic fibrosis (CF) patients. However, only marginal improvement in lung function has been observed, probably because DNase I requires exogenous divalent cations and is inhibited by G-actin, abundant in the sputum. In contrast, DNase II is unaffected by divalent cations or actin and may therefore be a superior therapeutic. We are studying the structure and activity of DNase II to evaluate its potential as a CF mucolytic agent. Purified DNase II from porcine spleen and **human** liver was previously shown to contain two thiol cross-linked peptides derived by posttranslational processing of a single polypeptide with formula weight of 39.6 kDa. Commercial DNase II contains a peptide that begins at amino acid 108, consistent with this structure. However, screening of several cell types and secretions in this laboratory has failed to demonstrate this processing. Rather, these systems show a 51 kDa protein that is reduced in size to 40 kDa upon incubation of cells with the N-glycosylation inhibitor tunicamycin. The protein contains at least one thiol cross-link. Similar results are obtained upon transient transfection of DNase II into several cell lines. Various truncated forms of the protein remain unglycosylated and inactive. These results suggest that the **human** DNase II protein is formed from one contiguous polypeptide chain, heavily glycosylated, which requires the signal peptide sequence for proper processing. This knowledge is important for production and evaluation of its therapeutic potential for CF.

L9 ANSWER 9 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:734130 HCAPLUS
DOCUMENT NUMBER: 134:38759
TITLE: TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix
AUTHOR(S): Yu, Wei-Hsuan; Yu, Shuan-Su C.; Meng, Qi; Brew, Keith; Woessner, J. Frederick, Jr.
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, 33101, USA
SOURCE: Journal of Biological Chemistry (2000), 275(40), 31226-31232
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Of the four known tissue inhibitors of metalloproteinases (TIMPs), TIMP-3 is distinguished by its tighter binding to the extracellular matrix. The present results show that glycosaminoglycans such as heparin, heparan sulfate, chondroitin sulfates A, B, and C, and sulfated compds. such as suramin and pentosan efficiently extract TIMP-3 from the postpartum rat uterus. Enzymic treatment by heparinase III or chondroitinase ABC also releases TIMP-3, but neither one alone gives complete release. Confocal microscopy shows co-localization of heparan sulfate and TIMP-3 in the endometrium subjacent to the lumen of the uterus. Immunostaining of TIMP-3 is lost upon **digestion** of tissue sections with heparinase III and chondroitinase ABC. The N-terminal domain of **human** TIMP-3 was expressed and found to bind to heparin with affinity similar to that of full-length mouse TIMP-3. The A and B β-strands of the N-terminal domain of TIMP-3 contain two potential heparin-binding sequences rich in lysine and arginine; these strands should form a double track on the outer surface of TIMP-3. Synthetic peptides corresponding to segments of these two strands compete for heparin in the **DNase**

II binding assay. TIMP-3 binding may be important for the cellular regulation of activity of the matrix metalloproteinases.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 10 OF 41 MEDLINE on STN
ACCESSION NUMBER: 2000127901 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10660581
TITLE: Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7).
AUTHOR: Yu W H; Woessner J F Jr
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101, USA.
CONTRACT NUMBER: AR-16940 (NIAMS)
SOURCE: Journal of biological chemistry, (2000 Feb 11) 275 (6) 4183-91.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000327
Last Updated on STN: 20000327
Entered Medline: 20000316
AB Many matrix metalloproteinases (MMPs) are tightly bound to tissues; matrilysin (MMP-7), although the smallest of the MMPs, is one of the most tightly bound. The most likely docking molecules for MMP-7 are heparan sulfate proteoglycans on or around epithelial cells and in the underlying basement membrane. This is established by extraction experiments and confocal microscopy. The enzyme is extracted from homogenates of postpartum rat uterus by heparin/heparan sulfate and by heparinase III treatment. The enzyme is colocalized with heparan sulfate in the apical region of uterine glandular epithelial cells and can be released by heparinase **digestion**. Heparan sulfate and MMP-7 are expressed at similar stages of the rat estrous cycle. The strength of heparin binding by recombinant rat proMMP-7 was examined by affinity chromatography, affinity coelectrophoresis, and homogeneous enzyme-based binding assay; the K(D) is 5-10 nM. Zymographic measurement of MMP-7 activity is greatly enhanced by heparin. Two putative heparin-binding peptides have been identified near the C- and N-terminal regions of proMMP-7; however, molecular modeling suggests a more extensive binding track or cradle crossing multiple peptide strands. Evidence is also found for the binding of MMP-2, -9, and -13. Binding of MMP-7 and other MMPs to heparan sulfate in the extracellular space could prevent loss of secreted enzyme, provide a reservoir of latent enzyme, and facilitate cellular sensing and regulation of enzyme levels. Binding to the cell surface could position the enzyme for directed proteolytic attack, for activation of or by other MMPs and for regulation of other cell surface proteins. Dislodging MMPs by treatment with compounds such as heparin might be beneficial in attenuating excessive tissue breakdown such as occurs in cancer metastasis, arthritis, and angiogenesis.

L9 ANSWER 11 OF 41 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2000457895 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10903447
TITLE: **Deoxyribonuclease II**: structure and chromosomal localization of the **murine** gene, and comparison with the genomic structure of the **human** and three C. elegans homologs.
AUTHOR: Krieser R J; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 03755, Hanover, NH, USA.

CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Gene, (2000 Jul 11) 252 (1-2) 155-62.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF190459; GENBANK-AF220525; GENBANK-AF220526
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20001005
Last Updated on STN: 20001005
Entered Medline: 20000925

AB **Deoxyribonuclease II** (DNase II) has been implicated in diverse functions including degradation of foreign DNA, genomic instability, and in mediating the DNA **digestion** associated with apoptosis. The production of a mouse deleted for DNase II would clearly help to discriminate these functions. We have cloned and sequenced the mouse gene encoding DNase II. It was found to have a similar intron/exon structure to the **human** gene, although introns 3 and 5 are considerably shorter. The gene is located on mouse chromosome 8. The order of genes at this locus is mGCDH, mEKLF, mDNase II, mSAST, which is the same order that these genes are found on **human** chromosome 19. The GenBank database contains incorrect expressed sequence tags (ESTs) for the 3' end of the mouse mRNA. Furthermore, the gene structure of two of the three homologs in C. elegans is also incorrectly predicted in the database. We have established the correct intron/exon structure for these genes and show the conserved sequence and structure of the C. elegans, **murine** and **human** genes.

L9 ANSWER 12 OF 41 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999410119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10482393
TITLE: Some properties of alkaline DNases of tentacles of actinia Radianthus macrodactylus and their hemolytic activity.
AUTHOR: Gaphurov J M; Bulgakov A A; Galkin V V; Rasskazov V A
CORPORATE SOURCE: Pacific Institute of Bioorganic Chemistry, Far Eastern Division of Russian Academy of Sciences, Vladivostok.
SOURCE: Toxicon : official journal of the International Society on Toxinology, (1999 Nov) 37 (11) 1591-604.
Journal code: 1307333. ISSN: 0041-0101.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991025

AB Two alkaline DNases of tentacles of actinia Radianthus macrodactylus, referred to as alk DNase I and alk DNase II, respectively, have been purified up to apparent homogeneity with consecutive column ion exchange chromatography and gel filtration. Both enzymes have a lot of common properties, such as the ability to hydrolyze very effectively p-nitrophenyl-5'-TMP and heat-denatured DNA. They both have no preferential specificity to the sugar component of the nucleic acids and effectively **digest** ribopolymers. Their ability to hydrolyze supercoiled DNA of the pBR322 plasmid and linear DNA of the lambda phage by "miscellaneous" exo- and endonucleolytic types of attack and to produce nucleosides, nucleotides and short oligonucleotides suggests their similarity with phosphodiesterase I (5'-exonuclease, oligonucleate 5'-nucleotidohydrolase; E.C. 3.1.4.1), isolated from rattle snake Crotalus adamenteus venom. Alk DNase II has been revealed to have some uncommon properties, such as phosphomonoesterase and hemolytic activities. The

protein causes a very potent lysis of **human** and rabbit erythrocytes. The ability of alk DNase II to precipitate some components of normal **human** and rabbit blood serum as well as the inhibition of this reaction by fucose but not by another monosaccharides suggest the enzyme to have a lectin-like activity. The appearance of only one protein band during electrophoresis of alk DNase II in denaturation conditions suggests that all activities are inherent to the same molecule of protein. The possible role of alkaline DNases in the toxic effect of burning by actinia tentacles is discussed.

L9 ANSWER 13 OF 41 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1999310942 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10381642
TITLE: Cleavage and nuclear translocation of the caspase 3 substrate Rho GDP-dissociation inhibitor, D4-GDI, during apoptosis.
AUTHOR: Krieser R J; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: CA09658 (NCI)
CA23108 (NCI)
CA50224 (NCI)
SOURCE: Cell death and differentiation, (1999 May) 6 (5) 412-9. Journal code: 9437445. ISSN: 1350-9047.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990921
Last Updated on STN: 20000303
Entered Medline: 19990909
AB While investigating endonucleases potentially involved in apoptosis, an antisera was raised to bovine **deoxyribonuclease II**, but it recognized a smaller protein of 26 kDa protein in a variety of cell lines. The 26 kDa protein underwent proteolytic cleavage to 22 kDa concomitantly with DNA **digestion** in cells induced to undergo apoptosis. Sequencing of the 26 kDa protein identified it as the Rho GDP-dissociation inhibitor D4-GDI. Zinc, okadaic acid, calyculin A, cantharidin, and the caspase inhibitor z-VAD-fmk, all prevented the cleavage of D4-GDI, DNA **digestion**, and apoptosis. The 26 kDa protein resided in the cytoplasm of undamaged cells, whereas following cleavage, the 22 kDa form translocated to the nucleus. **Human** D4-GDI, and D4-GDI mutated at the caspase 1 or caspase 3 sites, were expressed in Chinese hamster ovary cells which show no detectable endogenous D4-GDI. Mutation at the caspase 3 site prevented D4-GDI cleavage but did not inhibit apoptosis induced by staurosporine. The cleavage of D4-GDI could lead to activation of Jun N-terminal kinase which has been implicated as an upstream regulator of apoptosis in some systems. However, the results show that the cleavage of D4-GDI and translocation to the nucleus do not impact on the demise of the cell.

L9 ANSWER 14 OF 41 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-06794 BIOTECHDS
TITLE: **Human** and cattle **deoxyribonuclease-II** enzyme and encoding cDNA; recombinant DNA-ase-II preparation by vector expression in host cell, antisense oligonucleotide and antibody, used for cancer or autoimmune disease diagnosis or therapy, etc.
AUTHOR: Eastman A; Krieser R
PATENT ASSIGNEE: Dartmouth-Coll.
LOCATION: Hanover, NH, USA.
PATENT INFO: WO 9816659 23 Apr 1998

APPLICATION INFO: WO 1997-US18262 9 Oct 1997
PRIORITY INFO: US 1996-28539 15 Oct 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-251301 [22]
AB An isolated DNA-ase-II (EC-3.1.22.1) and cDNA encoding the enzyme are claimed. Also claimed are vectors containing the DNA; an antibody against the enzyme; and antisense oligonucleotides targeted to a DNA or mRNA encoding the enzyme. **Human** (1,915 bp) and cattle (927 bp) DNA sequences encoding 365 and 276 amino acid protein sequences are specified. The enzyme may be used to **digest** DNA, e.g. in the **human** lung sputum of cystic fibrosis patients to reduce sputum viscosity. Antibodies raised against the protein may be used diagnostically to determine apoptotic stages in selected cells by contacting the cells with the antibody, detecting binding with DNA-ase-II and determining DNA-ase-II levels (claimed). Identified inhibitors may be used to prevent diseases related to enhanced chromosomal rearrangement, e.g. cancers and autoimmune disorders. Vectors containing the DNA may be used to induce cell apoptosis, e.g. tumor cells and the antisense oligonucleotide may be administered to cells to inhibit DNA-ase-II expression, e.g. to reduce chromosome instability associated with cancer. (29pp)

L9 ANSWER 15 OF 41 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1999030349 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9812984
TITLE: The cloning and expression of **human** **deoxyribonuclease II**. A possible role in apoptosis.
AUTHOR: Krieser R J; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03655, USA.
CONTRACT NUMBER: CA09658 (NCI)
CA23108 (NCI)
CA50224 (NCI)
SOURCE: Journal of biological chemistry, (1998 Nov 20) 273 (47) 30909-14.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF047016; GENBANK-AF047017
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981221
AB We have previously implicated **deoxyribonuclease II** (DNase II) as an endonuclease responsible for DNA **digestion** during apoptosis. The full-length **human** cDNA has now been cloned. The cDNA contains an open reading frame of 1078 bases coding for a 40-kDa protein. This protein is 10 kDa larger than commercially supplied enzyme, which has been proteolytically cleaved at an internal aspartate residue. The gene is located at chromosome 19p13.2, and has no significant homology to other **human** proteins, but has >30% identity to three predicted genes in Caenorhabditis elegans. To determine whether overexpression of DNase II induces apoptosis in Chinese hamster ovary cells, the cDNA was cotransfected with a plasmid encoding green fluorescent protein. Within 24 h, a significant proportion of green fluorescent protein-positive cells contained condensed chromatin, whereas vector-only controls remained viable. Considering that DNase II is normally active only at low pH, it was surprising that transfection induced chromatin condensation. To confirm that transfection was not activating another endonuclease, cells were incubated with the caspase

inhibitor benzyloxycarbonyl-Val-Ala-Asp-(O-methyl)-fluoromethylketone; this failed to inhibit chromatin condensation induced by DNase II. These results demonstrate that DNase II acts downstream of caspase activation and that it may be activated by an as yet unknown mechanism to induce DNA **digestion** during apoptosis.

L9 ANSWER 16 OF 41 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:17326 BIOSIS
DOCUMENT NUMBER: PREV199900017326
TITLE: Identification of the three non-identical subunits constituting **human deoxyribonuclease II**.
AUTHOR(S): Takeshita, Haruo; Yasuda, Toshihiro; Iida, Reiko; Nakajima, Tamiko; Hosomi, Osamu; Nakashima, Yoshimitsu; Mori, Shinjiro; Nomoto, Hiroshi; Kishi, Koichiro [Reprint author]
CORPORATE SOURCE: Dep. Legal Med., Gunma Univ. Sch. Med., Maebashi 371-8511, Japan
SOURCE: FEBS Letters, (Nov. 27, 1998) Vol. 440, No. 1-2, pp. 239-242. print.
CODEN: FEBLAL. ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Jan 1999
Last Updated on STN: 20 Jan 1999
AB We purified DNase II from **human** liver to apparent homogeneity. The N-terminal amino acid sequences of each of three components constituting the purified mature enzyme were then separately determined by automatic Edman degradation. A combination of this chemical information and the previously reported nucleotide sequence of the cDNA encoding **human** DNase II (Yasuda et al. (1998) J. Biol. Chemical 273, 2610-2626) allowed detailed elucidation of the enzyme's subunit structure: **human** DNase II was composed of three non-identical subunits, a propeptide, proprotein and mature protein, following a signal peptide. Expression analysis of a series of deletion mutants derived from the cDNA of DNase II in COS-7 cells suggested that although a single large precursor protein may not be necessary for proteolytic maturation, the propeptide region L17-Q46 may play an essential role in generating the active form of the enzyme.

L9 ANSWER 17 OF 41 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1998:30088 BIOSIS
DOCUMENT NUMBER: PREV199800030088
TITLE: Intracellular acidification is associated with, but not required for caspase activation, DNA fragmentation or apoptosis.
AUTHOR(S): Reynolds, Jason E.; Wolf, Chad M.; Eastman, Alan [Reprint author]
CORPORATE SOURCE: Dep. Pharmacol., Dartmouth Med. Sch., Hannover, NH 03755, USA
SOURCE: International Journal of Oncology, (Dec., 1997) Vol. 11, No. 6, pp. 1241-1246. print.
ISSN: 1019-6439.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Jan 1998
Last Updated on STN: 14 Jan 1998
AB Apoptosis is characterized by DNA **digestion** mediated by either a Ca2+/Mg2+-dependent endonuclease or the acid-activated **deoxyribonuclease II** (DNase II). However, DNA **digestion** frequently does not correlate with changes in Ca2+ whereas intracellular acidification is a consistent marker of apoptosis. To confirm the role of low pH in regulating DNA **digestion**, ML-1

cells were damaged with etoposide then incubated at various extracellular pH (pHe). When pHe was 8.1, DNA **digestion** still occurred, and intracellular pH still decreased but only to 7.2, a pH at which DNase II is inactive. In contrast, low pH, inhibited the DNA **digestion** and apoptosis induced by etoposide. An upstream event in apoptosis is the activation of proteases known as caspases. The activity of caspases was inhibited at low pHe demonstrating that the pHsensitive step is upstream of caspase action. Similar results have been obtained in other models of apoptosis. Hence, both DNase II and Ca2+/Mg2+-dependent endonuclease appear unlikely to cause DNA **digestion** in apoptosis, unless their ion dependence is modified by, for example, proteolytic cleavage.

L9 ANSWER 18 OF 41 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 97109426 EMBASE
DOCUMENT NUMBER: 1997109426
TITLE: Utilization of an in vitro assay to evaluate chromatin degradation by candidate apoptotic nucleases.
AUTHOR: Hughes F.M. Jr.; Cidlowski J.A.
CORPORATE SOURCE: F.M. Hughes Jr., NIEHS, PO Box 12233, MD E2-02, Research Triangle Park, NC 27709, United States. Hughes4@niehs.nih.gov
SOURCE: Cell Death and Differentiation, (1997) 4/3 (200-208). Refs: 49
ISSN: 1350-9047 CODEN: CDDIEK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 001 Anatomy, Anthropology, Embryology and Histology
021 Developmental Biology and Teratology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Apoptosis is commonly associated with the catabolism of the genome in the dying cell. The chromatin degradation occurs in essentially two forms: (1) internucleosomal DNA cleavage to generate oligonucleosomal-length fragments (180-200 bp and multiples thereof), and (2) cleavage of higher order chromatin structures to generate .simeq. 30-50 Kb fragments. To investigate this component of apoptosis and identify the nuclease(s) responsible, we have developed and utilized an in vitro assay that recapitulates the genomic destruction seen during apoptosis in vivo and allows the simultaneous analysis of both forms of DNA degradation from the same sample. Using this assay we evaluated the **digestion** patterns of several candidate apoptotic nucleases: DNase I, DNase II, and cyclophilin (NUC18) as well as the bacterial enzyme micrococcal nuclease (not thought to be involved in apoptosis). Chromatin degraded by DNase I formed a smear of DNA on conventional static-field agarose gels and .simeq. 30-50 Kb DNA fragments on pulsed field gels. In contrast, DNase II, at a physiologically relevant pH, had no effect on the integrity of HeLa chromatin in either analysis. Similar to DNase I, cyclophilin C produced only .simeq. 30-50 Kb DNA fragments but did not generate internucleosomal fragments. In contrast, micrococcal nuclease generated both oligonucleosomal and .simeq. 30-50 Kb DNA fragments. Nuclear extracts from glucocorticoid-treated apoptotic thymocytes generated oligonucleosomal DNA fragments and the larger .simeq. 30-50 Kb DNA fragments, fully recapitulating both types of apoptotic DNA degradation. Previously, differential sensitivity of nucleases to inhibition by Zn2+ was used to argue that two distinct enzymes mediate .simeq. 30-50 Kb DNA cleavage and internucleosomal DNA degradation. While, the nuclease activity present in thymocyte nuclear extracts was differentially sensitive to inhibition by Zn2+ during short term incubations it was not during prolonged **digestions**, suggesting that differences in DNA detection are likely to account for previous results, together our studies show that none of the nucleases commonly associated with apoptosis could fully recapitulate the DNA degradation seen in vivo.

L9 ANSWER 19 OF 41 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 97:108926 SCISEARCH
THE GENUINE ARTICLE: WE588
TITLE: Zinc inhibits apoptosis upstream of ICE/CED-9 proteases rather than at the level of an endonuclease
AUTHOR: Wolf C M; Morana S J; Eastman A (Reprint)
CORPORATE SOURCE: DARTMOUTH COLL, SCH MED, DEPT PHARMACOL & TOXICOL, HANOVER, NH 03755 (Reprint); DARTMOUTH COLL, SCH MED, DEPT PHARMACOL & TOXICOL, HANOVER, NH 03755
COUNTRY OF AUTHOR: USA
SOURCE: CELL DEATH AND DIFFERENTIATION, (FEB 1997) Vol. 4, No. 2, pp. 125-129.
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS.
ISSN: 1350-9047.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Apoptosis is commonly associated with DNA **digestion**, but it remains controversial as to which endonuclease is involved. The ability of zinc to inhibit DNA **digestion** in intact cells, and inhibit a Ca2+/Mg2+-dependent endonuclease in cell lysates, has been used frequently to suggest this is the endonuclease involved. However, zinc has many other effects on cells, and here it is shown that zinc also prevents many upstream events in apoptosis. These studies were performed in **human** ML-1 cells following incubation with etoposide. During apoptosis, these cells undergo intracellular acidification, increased accumulation of Hoechst 33342, DNA **digestion** and chromatin condensation. Zinc inhibited all of these events. An upstream event in apoptosis is activation of ICE/CED-3 proteases which is commonly observed as proteolysis of a substrate protein, poly(ADP-ribose) polymerase (PARP). The ICE/CED-3 proteases are themselves activated by proteolysis, and this was detected here by cleavage of one family member CPP32. Zinc prevented cleavage of both CPP32 and PARP. We recently demonstrated that dephosphorylation of the retinoblastoma susceptibility protein Rb was a marker of an event even further upstream in apoptosis; zinc was also found to inhibit Rb dephosphorylation. Therefore, zinc must protect cells at a very early step in the apoptotic pathway, and not as a direct inhibitor of an endonuclease.

L9 ANSWER 20 OF 41 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 96:707982 SCISEARCH
THE GENUINE ARTICLE: VJ466
TITLE: AN APOPTOTIC ENDONUCLEASE ACTIVATED EITHER BY DECREASING PH OR BY INCREASING CALCIUM
AUTHOR: COLLINS M K L (Reprint); FURLONG I J; MALDE P; ASCASO R; OLIVER J; RIVAS A L
CORPORATE SOURCE: CRC, CTR CELL & MOL BIOL, CHESTER BEATTY LABS, 237 FULHAM RD, LONDON SW3 6JB, ENGLAND (Reprint); CSIC, INST PARASITOL & BIOMED, GRANADA 18001, SPAIN
COUNTRY OF AUTHOR: ENGLAND; SPAIN
SOURCE: JOURNAL OF CELL SCIENCE, (SEP 1996) Vol. 109, Part 9, pp. 2393-2399.
ISSN: 0021-9533.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB DNA fragmentation in isolated nuclei from the **murine** IL3-dependent bone marrow cell line BAF3 could be stimulated either by decreasing pH below 6.5 or by adding mu M calcium at neutral pH, An endonuclease which could also be stimulated either by a decrease in pH, to 6.5, or by the presence of mu M calcium at neutral pH, was purified 10(4)-fold from nuclei of BAF3 cells. **Digestion** of DNA with the purified enzyme resulted in 5'-terminal hydroxyl and 3'-terminal phosphate ends, These characteristics are distinct from those described for other mammalian endonucleases. The possible role of this enzyme in genome **digestion** during apoptosis is discussed.

L9 ANSWER 21 OF 41 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 94:510197 SCISEARCH
THE GENUINE ARTICLE: PC065
TITLE: DETECTION OF DEOXYRIBONUCLEASE-I AND **DEOXYRIBONUCLEASE-II** (DNASE-I AND DNASE-II) ACTIVITIES IN REPRODUCTIVE-ORGANS OF MALE RABBITS
AUTHOR: TAKESHITA H; YASUDA T; NADANO D; TENJO E; SAWAZAKI K; IIDA R; KISHI K (Reprint)
CORPORATE SOURCE: FUKUI MED SCH, DEPT LEGAL MED, MATSUOKA CHO, FUKUI 91011, JAPAN (Reprint); FUKUI MED SCH, DEPT LEGAL MED, FUKUI 91011, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: INTERNATIONAL JOURNAL OF BIOCHEMISTRY, (AUG 1994) Vol. 26, No. 8, pp. 1025-1031.
ISSN: 0020-711X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 27

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB 1. Deoxyribonucleases (DNases) I and II activities in 13 different organs and body fluids from healthy male rabbits were measured using the single radial enzyme diffusion method.

2. We now show that testis, epididymis, ampulla, seminal vesicle, vesicular gland, prostate, and semen have both of the DNases I and II activities, whereas spermatozoa do not.

3. DNase I activities were highest in epididymis and seminal vesicle, whereas DNase II activities were highest in epididymis and prostate among the reproductive organs.

4. The presence of these two enzyme activities outside the **digestive** system suggests that they have another biological function in addition to their **digestive** roles.

L9 ANSWER 22 OF 41 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 94349554 EMBASE
DOCUMENT NUMBER: 1994349554
TITLE: Evidence for direct anti-heparin-sulphate reactivity in sera of SLE patients.
AUTHOR: Pirner K.; Rascu A.; Nurnberg W.; Rubbert A.; Kalden J.R.; Manger B.
CORPORATE SOURCE: Inst. Clin. Immunology Rheumatology, Department of Medicine III, Medical School Erlangen, Krankenhausstrasse 12,D-91054 Erlangen, Germany
SOURCE: Rheumatology International, (1994) 14/4 (169-174).
ISSN: 0172-8172 CODEN: RHINDE
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
026 Immunology, Serology and Transplantation
028 Urology and Nephrology

031 Arthritis and Rheumatism
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Recently it has been suggested that anti-dsDNA antibodies (Abs) promote tissue damage in systemic lupus erythematosus (SLE) by cross-reactivity with highly negatively charged tissue components such as heparan sulphate (HS), the major glycosaminoglycan of the glomerular basement membrane (GBM). Other authors, however, support the theory of DNA-anti-dsDNA immune complex deposition in situ. To further elucidate the possible role of HS antibodies, we developed a new ELISA system with heparan sulphate bound to solid phase. SLE patients (n = 40) showed a higher reactivity against HS (mean = 28.4, SD = 34.3) as compared to normal donors (n = 28, mean = 15.2, SD = 6.3) and patients with rheumatoid arthritis (n = 35, mean = 14.3, SD = 6.4). The addition of native dsDNA or HS to SLE sera was followed by a dose-dependent reduction in anti-HS reactivity. In contrast, in an anti-dsDNA ELISA, no reduction was observed when HS was added to SLE sera. An increase in reactivity was observed when SLE sera with and without a prior incubation with dsDNA were **digested** with DNAse I or II. After the purification of serum samples by protein A sepharose under dissociative conditions, seven out of eight SLE patients showed an increase in anti-HS reactivity. No correlation of the anti-HS Abs was found with organ involvement or other serological parameters. We concluded, that there is evidence for a direct anti-HS Ab reactivity in SLE sera. A part of these antibodies seems to show low avidity anti-dsDNA cross-reactivity.

L9 ANSWER 23 OF 41 EMBASE
on STN
ACCESSION NUMBER: 94227570 EMBASE
DOCUMENT NUMBER: 1994227570
TITLE: The inhibition of etoposide-induced apoptosis by zinc is associated with modulation of intracellular pH.
AUTHOR: Morana S.; Li J.; Springer E.W.; Eastman A.
CORPORATE SOURCE: Department of Pharmacology, 7650 Remsen, Dartmouth Medical School,Hanover, NH 03755, United States
SOURCE: International Journal of Oncology, (1994) 5/2 (153-158). ISSN: 1019-6439 CODEN: IJONES
COUNTRY: Greece
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Apoptosis is associated with DNA fragmentation, usually as a result of the activation of an endonuclease that **digests** chromatin DNA between the nucleosomes. The identity of the endonuclease is important for understanding the regulation of apoptosis. A Ca2+/Mg2+-dependent endonuclease is often cited as the critical endonuclease. One inhibitor that has been used to implicate this endonuclease is zinc, which inhibits the endonuclease in vitro and also inhibits apoptosis. **Deoxyribonuclease II** is an alternate endonuclease that could be involved in apoptosis, yet it is not inhibited by zinc. **Deoxyribonuclease II** is activated by intracellular acidification which occurs during apoptosis. The current experiments show that zinc inhibits the intracellular acidification associated with apoptosis which may be an alternate means by which it inhibits DNA **digestion**. Hence zinc appears to inhibit both endonucleases in intact cells, so can not be used to specifically implicate either.

L9 ANSWER 24 OF 41 EMBASE
on STN DUPLICATE 8
ACCESSION NUMBER: 93156838 EMBASE
DOCUMENT NUMBER: 1993156838

TITLE: Etoposide-induced apoptosis in **human** HL-60 cells is associated with intracellular acidification.
AUTHOR: Barry M.A.; Reynolds J.E.; Eastman A.
CORPORATE SOURCE: Department of Pharmacology, Dartmouth Medical School,Hanover, NH 03755-3835, United States
SOURCE: Cancer Research, (1993) 53/10 (2349-2357).
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Apoptosis is a pathway of cell death characterized by internucleosomal **digestion** of genomic DNA. Such DNA **digestion** can be induced by both physiological stimuli and cytotoxic treatment with many anticancer agents. This **digestion** has generally been considered to be mediated by a Ca2+/Mg2+-dependent endonuclease that is activated by increases in intracellular Ca2+. However, we suggest that an alternate endonuclease, DNase II, may be a more likely candidate. In these studies, apoptosis was induced in **human** HL-60 cells by a 30-min incubation with the topoisomerase II inhibitor etoposide. DNA **digestion** characteristic of apoptosis began within 3 h of removal of etoposide. Morphological indication of apoptosis was observed concurrently. Only about 20% of the cells underwent apoptosis at this time; these appeared to be cells in S phase at the time of etoposide treatment. The remainder of the cells progressed to the G2 phase and arrested there for at least 48 h. Intracellular Ca2+ and pH were measured in individual cells by flow cytometry. No changes in intracellular Ca2+ were observed, but an acidification of up to 1 pH unit occurred in about 15% of the cells and correlated with the time course of appearance of DNA **digestion**. Cells were sorted on the basis of intracellular pH and only the acidic cells showed the morphology and DNA **digestion** characteristic of apoptosis. These results demonstrate the involvement of DNase II in apoptotic DNA **digestion** and suggest mechanisms of pH homeostasis as regulators of apoptosis.

L9 ANSWER 25 OF 41 MEDLINE on STN
ACCESSION NUMBER: 93339604 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8339953
TITLE: DNase activity in **murine** lenses: implications for cataractogenesis.
AUTHOR: Graw J; Liebstein A
CORPORATE SOURCE: GSF-Forschungszentrum fur Umwelt und Gesundheit, Institut fur Saugetiergenetik, Neuherberg, Germany.
SOURCE: Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, (1993 Jun) 231 (6) 354-8.
Journal code: 8205248. ISSN: 0721-832X.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930917
Last Updated on STN: 19930917
Entered Medline: 19930830

AB In **murine** lens extracts a Mg(2+)-dependent DNase activity was found and characterized with respect to its ionic conditions. The lenticular DNase can be clearly distinguished from DNaseII. Only a moderate DNase activity is detectable in intact nuclei of lens cells from 1-day-old mice, but DNase is obviously present with high activity in lens cell nuclei from 7-day-old mice. During this time, when **murine**

eyes are not yet open, and the fiber cell nuclei including the nuclear membrane remain to be completely **digested**, only weak activity can be detected in cytosolic lens extracts. In three allelic dominant mice mutants exhibiting hereditary cataracts the DNase activity is inhibited. The decrease of DNase activity follows the same directionality (Cat-2ns > Cat-2no > Cat-2t) as the decrease in the relative content of water soluble lens proteins, which might be used as a rough indicator for the severity of cataractogenesis. Both trends are highly significant ($P < 0.0001$).

L9 ANSWER 26 OF 41 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 92360026 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1323291
TITLE: Endonuclease activation during apoptosis: the role of cytosolic Ca2+ and pH.
AUTHOR: Barry M A; Eastman A
CORPORATE SOURCE: Department of Pharmacology, Dartmouth Medical School, Hanover, NH 03755-3835.
CONTRACT NUMBER: CA 23108 (NCI)
CA 50224 (NCI)
SOURCE: Biochemical and biophysical research communications, (1992 Jul 31) 186 (2) 782-9.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920925
Last Updated on STN: 19970203
Entered Medline: 19920904
AB An axiom of apoptosis is that increases in cytosolic Ca2+ activate a Ca2+/Mg(2+)-dependent endonuclease. However, when HL-60 **human** promyelocytic leukemia cells were incubated with the Ca2+ ionophore ionomycin in varied extracellular Ca2+, DNA **digestion** was independent of extracellular Ca2+. Under these conditions, intracellular Ca2+ concentrations did not correlate with the observed DNA **digestion**. In contrast, intracellular acidification correlated well with DNA **digestion**. These data indicate that increased intracellular Ca2+ is not the primary signal for endonuclease activation in all forms of apoptosis, but that intracellular acidification may be involved. The observed intracellular acidification is consistent with the involvement of **deoxyribonuclease II** in apoptosis.

L9 ANSWER 27 OF 41 MEDLINE on STN
ACCESSION NUMBER: 89051786 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2461254
TITLE: Nerve growth factor receptors in chromatin of melanoma cells, proliferating melanocytes, and colorectal carcinoma cells in vitro.
AUTHOR: Rakowicz-Szulczynska E M; Herlyn M; Koprowski H
CORPORATE SOURCE: Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.
CONTRACT NUMBER: CA-10815 (NCI)
CA-25874 (NCI)
CA-29200 (NCI)
SOURCE: Cancer research, (1988 Dec 15) 48 (24 Pt 1) 7200-6.
Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198901
ENTRY DATE: Entered STN: 19900308

Last Updated on STN: 19970203
Entered Medline: 19890109

AB Nuclear localization of nerve growth factor (NGF) in HS 294 melanoma cells and SW 707 colorectal carcinoma was determined by indirect immunofluorescence staining and by cell fractionation. NGF receptors were immunoprecipitated from the EcoRI-**digested** chromatin of HS 294 melanoma cells, of melanocytes proliferating in the presence of 12-O-tetradecanoylphorbol-13-acetate, and of SW 707 colorectal carcinoma cells, using a monoclonal antibody to the Mr 75,000 cell surface NGF receptor. Melanoma cells expressed a receptor species with a molecular weight of 230,000. Proliferating melanocytes expressed a small amount of Mr 230,000 receptor, whereas colorectal carcinoma cells expressed a Mr 35,000 receptor. Scatchard analysis indicated one type of NGF chromatin binding site in HS 294 cells with KD = 241 pM but two types of binding sites in chromatin of SW 707 cells with KD = 333 and 1718 pM, respectively. Both the Mr 230,000 and the 35,000 receptor species were tightly bound to DNase II-sensitive regions, which became DNase II-insensitive after nerve growth factor binding. [125I]NGF was detected in the chromatin in nondegraded form. Chromatin binding of NGF inhibited RNA synthesis and cell proliferation.

L9 ANSWER 28 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:419428 HCAPLUS
DOCUMENT NUMBER: 105:19428
TITLE: Chromatin binding of epidermal growth factor, nerve growth factor, and platelet-derived growth factor in cells bearing the appropriate surface receptors
AUTHOR(S): Rakowicz-Szulczynska, Ewa M.; Rodeck, Ulrich; Herlyn, Meenhard; Koprowski, Hilary
CORPORATE SOURCE: Wistar Inst. Anat. Biol., Philadelphia, PA, 19104, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1986), 83(11), 3728-32
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The uptake and intracellular distribution of 125I-labeled EGF [62229-50-9], nerve growth factor [9061-61-4], and platelet-derived growth factor were analyzed in different cell lines that express or do not express the resp. surface receptors for these factors. After 1 h of incubation, all 3 growth factors were detected in the cytoplasmic fraction and in the nucleus, tightly bound to chromatin. The amount of chromatin-bound growth factors continued to increase during the incubation, and anal. at 48 h revealed each chromatin-bound labeled growth factor in a nondegraded form. After limited **digestion** of chromatin with **DNAse II** (10-20% **digested** sequences), specific release of all 3 growth factors was detected only after 1 h of incubation but not after 24 and 48 h, suggesting that the DNA regions involved in growth factor binding became nuclease resistant. Binding of labeled EGF and nerve growth factor to isolated chromatin was inhibited by monoclonal antibodies specific for the resp. growth factor receptor. Chromatin binding may represent an important step in the pathway of growth factor action.

L9 ANSWER 29 OF 41 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 87048742 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3022715
TITLE: Identification of NGF receptor in chromatin of melanoma cells using monoclonal antibody to cell surface NGF receptor.
AUTHOR: Rakowicz-Szulczynska E M; Koprowski H
CONTRACT NUMBER: CA-1085 (NCI)
CA-21124 (NCI)
CA-25874 (NCI)
+

SOURCE: Biochemical and biophysical research communications, (1986 Oct 15) 140 (1) 174-80.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198612
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19861204

AB A 230 KDa species of Nerve Growth Factor (NGF) receptor was immunoprecipitated from EcoRI-**digested** chromatin of melanoma cells using a monoclonal antibody to the 75 KDa cell surface NGF receptor. The chromatin NGF receptor was shown to exist tightly bound to DNase II-sensitive sequences which, upon growth factor binding, became resistant to DNase II **digestion**.

L9 ANSWER 30 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1984:203235 HCAPLUS
DOCUMENT NUMBER: 100:203235
TITLE: Cellular and molecular mechanisms of the bone marrow sparing effects of the glucose chloroethylnitrosourea chlorozotocin
AUTHOR(S): Byrne, P.; Tew, K.; Jemionek, J.; MacVittie, T.; Erickson, L.; Schein, P.
CORPORATE SOURCE: Vincent T. Lombardi Cancer Res. Cent., Georgetown Univ. Hosp., Washington, DC, USA
SOURCE: Blood (1984), 63(4), 759-67
CODEN: BLOOAW; ISSN: 0006-4971
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CCNU [13010-47-4] and other chloroethylnitrosourea anticancer agents in clin. use produce severe and cumulative bone marrow toxicity. Chlorozotocin [54749-90-5], a glucose analog, has demonstrated reduced hematol. toxicity while retaining full antitumor activity. The biochem.-pharmacol. properties of chlorozotocin and CCNU were compared in **human** bone marrow. After a 2-h incubation with a 0.1-mM drug concentration, total cellular uptake of chlorozotocin in whole marrow was 2.47 pmol/104 cells and was not much different compared to the uptake of 1.94 pmole/104 cells with CCNU. The quant. alkylation of bone marrow DNA by chlorozotocin, 22.8 pmole/mg DNA, was equivalent to that produced by CCNU, 22.9 pmole/mg DNA. Bone marrow was separated into 14 fractions by centrifugal elutriation. CCNU uptake was greater than that of chlorozotocin in 3 fractions that were primarily composed of lymphocytes, monocytes, and normoblasts. Chlorozotocin uptake was greater than CCNU in 6 fractions that contained primarily mature and immature myeloid cells as well as the highest CFU-GM activity. The 2 drugs produced a comparable degree of DNA strand breakage and DNA-protein crosslinking. DNA interstrand crosslinking was not found with either drug. The most significant finding of this study is the differences in the site of drug alkylation by chlorozotocin and CCNU in bone marrow chromatin. Endonuclease **digestions** with micrococcal nuclease, DNase I, and **DNAse II** showed nonrandom alkylation of specific regions of chromatin by the 2 drugs. CCNU demonstrated a preferential binding to the transcriptionally active regions of chloromatin, whereas chlorozotocin predominantly alkylated the transcriptionally inactive regions. Apparently, the lethal damage of nitrosourea alkylation in **human** bone marrow is principally expressed in transcriptionally active regions of chromatin.

L9 ANSWER 31 OF 41 MEDLINE on STN
ACCESSION NUMBER: 85002710 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6478794

TITLE: Release of ribonucleoprotein during **digestion** of rat testis chromatin with **deoxyribonuclease II** (3.1.4.6).
AUTHOR: Grimes S R Jr
CONTRACT NUMBER: HD11796 (NICHD)
SOURCE: Comparative biochemistry and physiology. B, Comparative biochemistry, (1984) 78 (3) 633-41.
Journal code: 2984730R. ISSN: 0305-0491.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198411
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 20021218
Entered Medline: 19841106

AB The composition of rat testis chromatin proteins in fractions produced by limited DNase II **digestion** followed by differential precipitation with MgCl2 has been studied. Over 50% of the acid-soluble proteins in the soluble chromatin fraction appeared to be quite similar to proteins which are associated with ribonucleoprotein (RNP) particles in HeLa cells. Although the ratios of the testis RNP protein components differed from those of HeLa RNP particles, the three major polypeptides were most similar to the HeLa components designated A2, B2, and C1. The soluble chromatin fraction was also enriched in the high mobility group proteins HMG1 and HMG2.

L9 ANSWER 32 OF 41 MEDLINE on STN
ACCESSION NUMBER: 83257382 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6307389
TITLE: Implications of a 5'-nucleotidase inhibitor in **human** leukemic cells for cellular aging and cancer.
AUTHOR: Sun A S; Holland J F; Lin K; Ohnuma T
SOURCE: Biochimica et biophysica acta, (1983 Jul 14) 762 (4) 577-84.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198309
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19980206
Entered Medline: 19830909

AB 5'-Nucleotidase activity of normal **human** embryonic lung fibroblasts (IMR-90) was found to be inhibited by the homogenates of seven different cell lines originated from patients with different kinds of leukemia and of fresh lymphocytes from a patient with Sezary syndrome (circulating T-cell lymphoma). About 97% of the inhibiting activity was found in the soluble fraction of RPMI 8402 cells, a cell line originated from the lymphocytes of a patient with acute lymphocytic leukemia. This inhibiting activity was not destroyed by dialysis, heating at 56 degrees C for 30 min, nor **digestion** with RNAase or DNAase. About 85% of the inhibiting activity was destroyed by **digestion** with papain at 37 degrees C for 1 h and it was destroyed completely by heating at 100 degrees C for 30 min. When the heated (56 degrees C for 30 min) soluble fraction of RPMI 8402 cells was mixed with the homogenate of IMR-90 cells, it had no effect on the activities of alkaline, neutral or acid phosphatases, nor of N-acetyl-beta-D-glucosaminidase or cytochrome c oxidase of IMR-90 cells. Preincubating the mixed samples for 1, 20 and 45 min, respectively, before adding the substrate, the heated soluble fraction of RPMI 8402 cells did not increase the percentage of inhibition for 5'-nucleotidase of the homogenate of IMR-90 cells. No inhibition of other enzyme activities was observed under similar conditions. These data

suggest that the inhibiting activity is due to a protein(s) that is not a protease. The inhibiting activity was found in a single peak after the soluble fraction was fractionated by Sephadex G-100 chromatography and sedimentation centrifugation. The molecular weight of the inhibitor was found to be approx. 35,000 by comparing its retention volume and sedimentation rate with those of proteins of known molecular weight. The present study suggest that the previously reported undetectability of 5'-nucleotidase in permanent cell lines could be due to the presence of a protein inhibitor for 5'-nucleotidase in these **human** leukemic cell lines. It also supports the hypothesis that the increased 5'-nucleotidase activity in normal senescent cells in vitro may be a control in cellular aging that is missing from leukemic cells in vitro.

L9 ANSWER 33 OF 41 MEDLINE on STN
ACCESSION NUMBER: 82274241 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6287433
TITLE: Chromosome-bound mitotic factors: release by endonucleases.
AUTHOR: Adlakha R C; Sahasrabuddhe C G; Wright D A; Lindsey W F; Smith M L; Rao P N
CONTRACT NUMBER: CA 11520 (NCI)
CA 27544 (NCI)
SOURCE: Nucleic acids research, (1982 Jul 10) 10 (13) 4107-17. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198210
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19970203
Entered Medline: 19821029

AB Additional evidence is presented to support our recently reported conclusion that the mitotic factors of mammalian cells, which induce germinal vesicle breakdown and chromosome condensation when injected into fully grown Xenopus laevis oocytes, are localized on metaphase chromosomes. Chromosomes isolated from mitotic HeLa cells were further purified on sucrose gradients and **digested** for varying periods with either the micrococcal nuclease or DNase II. At each time point of **digestion** the amount of mitotic factors released was determined by injecting a supernatant of these fractions, obtained by high-speed centrifugation, into oocytes. The amount of DNA rendered acid soluble under the conditions of **digestion** used was 3% ot 5% of the total chromosomal DNA. The extent of release of mitotic factors with both nucleases was estimated to be about 30% to 40% as evidenced by the reextraction of the undigested chromosomal pellet with 0.2 M NaCl. Similar results were obtained when nuclei from G2 cells were **digested** under identical conditions. The release of these chromosome-bound mitotic factors by mild **digestion** with these nucleases though only partial, clearly demonstrates that a significant proportion of these factors are localized on metaphase chromosomes.

L9 ANSWER 34 OF 41 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 82:66778 LIFESCI
TITLE: Micrococcal nuclease and DNase I **digestion** of DNA from aging **human** diploid cells.
AUTHOR: Dell'Orco, R.T.; Whittle, W.L.
CORPORATE SOURCE: Biomed. Div., Samuel Roberts Noble Found., Inc., Ardmore, OK 73401, USA
SOURCE: BIOCHEM. BIOPHYS. RES. COMMUN., (1982) vol. 107, no. 1, pp. 117-122.
DOCUMENT TYPE: Journal
FILE SEGMENT: N; G
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Nuclei prepared from confluent and mitotically arrested populations of **human** diploid fibroblast-like cells of different in vitro ages were subjected to **digestion** by micrococcal nuclease and DNase I. There was no age or culture state variation in the susceptibility of DNA to micrococcal nuclease **digestion**. There was, however, an age related inhibition of DNA **digestion** by DNase I in nuclei from older confluent but not older arrested cells. It is suggested that this is the result of an age related masking by nucleosome core histones which limits the accessibility of DNA to enzymatic activities in older confluent cells.

L9 ANSWER 35 OF 41 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 80227637 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6248503
TITLE: Purification and properties of **deoxyribonuclease II** from **human** urine.
AUTHOR: Murai K; Yamanaka M; Akagi K; Anai M
SOURCE: Journal of biochemistry, (1980 Apr) 87 (4) 1097-103. Journal code: 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198009
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19970203
Entered Medline: 19800923

AB The acid deoxyribonucleases [DNase II; EC 3.1.4.6] in **human** urine were purified approximately 400- to 500-fold by phosphocellulose chromatography, gel filtration on Sephadex G-75 and isoelectric focusing, with a total recovery of 22%. The enzymes were present in a least three forms with different isoelectric points, pHs 6.4, 6.6, and 6.8. However, other properties were essentially similar. The enzymes did not require divalent cations for activity, and the optimal pHs were at 5.1 to 5.3 in 33 mM acetate buffer. They had a molecular weight of around 36,000, as estimated by gel filtration on Sephadex G-75. The enzymes were endonucleases which hydrolyzed native, double-stranded DNA about 5 to 15 times faster than thermally denatured DNA. The products formed from native DNA were 3'-phosphoryl- and 5'-hydroxy-terminated oligonucleotides. The average chain length of the limit **digests** with these enzymes was approximately 11 to 15, and the major fragments were longer than pentanucleotides. The final preparations were free of nonspecific acid and alkaline phosphatases and phosphodiesterase, but contained contaminating ribonuclease activity.

L9 ANSWER 36 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1980:547176 HCAPLUS
DOCUMENT NUMBER: 93:147176
TITLE: Distribution of DNA repair and the extent of enzymatic DNA methylation in alkylated **human** lymphocytes carrying their DNA synthesis in the presence or absence of hydroxyurea
AUTHOR(S): Malec, Janina; Sawecka, Jadwiga; Kornacka, Ludwika
CORPORATE SOURCE: Dep. Biochem., Inst. Haematol., Warsaw, 00-957, Pol.
SOURCE: Biochemical and Biophysical Research Communications (1980), 95(1), 304-11
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The distribution of repair label into **DNase II** -sensitive and -resistant regions of chromatin in **human** lymphocytes exposed to nitrogen mustard indicates that in the regions most accessible to this enzyme the number of newly inserted label was .apprx.3.5-fold greater than that in resistant regions with only a slight

preference for the sequences considered to be transcriptionally active. In the course of **digestion**, this proportion becomes gradually lower. Pyrimidine tract anal. did not reveal significant differences between damaged and undamaged cells. In repair-inserted sequences ≤1/50-60 cytosines was methylated, whereas in undamaged lymphocytes .apprx.1/15 cytosines was modified. The presence or absence of hydroxyurea during the course of repair synthesis did not seem to affect any of the parameters studied.

L9 ANSWER 37 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1980:493180 HCAPLUS
DOCUMENT NUMBER: 93:93180
TITLE: Intragenomic distribution of 5-methylcytosine in various forms of **human** and **murine** leukemic cells
AUTHOR(S): Sawecka, J.; Kornacka, L.; Malec, J.
CORPORATE SOURCE: Inst. Hematol., Warsaw, 00-957, Pol.
SOURCE: Neoplasma (1980), 27(2), 187-91
CODEN: NEOLA4; ISSN: 0028-2685
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In **human** chronic myelogenic leukemia and acute leukemia leukocytes, phytohemagglutinin (PHA)-stimualted **human** lymphocytes, and **murine** L5178Y lymphoblasts cultured in various phases of growth, the general pattern of intragenomic 5-methylcytosine distribution was similar, with 2 preferentially methylated regions (the sequences fast reassocg. and rendered Mg++-soluble after **DNase II digestion** of nuclei). The most variable fraction, as regards the level of methylation, seemed to be DNA of the Mg++-soluble fraction of the **DNase II digest**, which in acute leukemia leukocytes, PHA-stimulated lymphocytes, and exponentially growing L5178Y cells contained .apprx. 2-fold higher proportions of methylated cytosines than did leukocytes of chronic myelogenic leukemia and L5178Y cells maintained at saturation d.

L9 ANSWER 38 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1979:572464 HCAPLUS
DOCUMENT NUMBER: 91:172464
TITLE: Heterogeneity of DNA methylation in **murine** L5178Y lymphoblasts
AUTHOR(S): Sawecka, J.; Kornacka, L.; Malec, J.
CORPORATE SOURCE: Dep. Biochem., Inst. Haematol., Warsaw, PL-00-957, Pol.
SOURCE: Experientia (1979), 35(9), 1166-7
CODEN: EXPEAM; ISSN: 0014-4754
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The highest rate of DNA methylation occurred in the MgCl2-soluble fraction of **DNase II-digested** L5178 leukemia cell chromatin and in the nucleolar and fast-reassocg. DNA fractions. The relation of the transcriptional activity of these DNA fractions to the rate of DNA methylation is discussed.

L9 ANSWER 39 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1976:572212 HCAPLUS
DOCUMENT NUMBER: 85:172212
TITLE: Specific herpes simplex virus-induced incorporation of 5-iodo-5'-amino-2',5'-dideoxyuridine into deoxyribonucleic acid
AUTHOR(S): Chen, Ming S.; Ward, David C.; Prusoff, William H.
CORPORATE SOURCE: Sch. Med., Yale Univ., New Haven, CT, USA
SOURCE: Journal of Biological Chemistry (1976), 251(16), 4833-8
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal
LANGUAGE: English
AB 5-Iodo-5'-amino-2',5'-dideoxyuridine (I) [56045-73-9] is a novel thymidine analog which inhibits herpes simplex virus, type 1 (HS-1 virus) replication in the absence of detectable host toxicity. When **murine**, simian, or **human** cells in culture were treated with I-125I for ≤24 hr, essentially none of the nucleoside became cell-associated In contrast, upon HS-1 virus infection significant radiolabel was detected in both nucleotide pools and in DNA. The major acid-soluble metabolite was I 5'-triphosphate [60658-58-4]. DNA from HS-1 virus-infected Vero cells labeled with thymidine-14C, 5-iodo-2'-deoxyuridine-125I (IdUrd), or I-125I was isolated by buoyant d. centrifugation and subjected to **digestion** by pancreatic DNase I, spleen **DNase II**, micrococcal nuclease, and spleen and venom phosphodiesterases. Anal. of the **digestion** products clearly indicates that I is incorporated internally into the DNA structure. DNA containing I therefore contains phosphoramidate (P-N) bonds, known to be extremely acid labile. The selective HS-1 virus-induced phosphorylation of I and its subsequent incorporation into DNA may account for the unique biol. activity of the I nucleoside.

L9 ANSWER 40 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1971:495247 HCAPLUS
DOCUMENT NUMBER: 75:95247
TITLE: Electron microscopic localization of acridine orange binding to DNA within **human** leukemic bone marrow cells
AUTHOR(S): Frenster, John H.
CORPORATE SOURCE: Sch. Med., Stanford Univ., Stanford, CA, USA
SOURCE: Cancer Research (1971), 31(8), 1128-33
CODEN: CNREA8; ISSN: 0008-5472
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The title technique applied to diagnostic samples from untreated patients with chronic myelogenous leukemia gives both resolution and chemical specificity higher than by microspectrofluorimetry. Acridine orange (I) binds to DNA only within the active extended part of the cell nucleus, as predicted from cited data. This reaction of 10-3M I after glutaraldehyde fixation, then **digestion** with **DNase** (**II**), occurs in all types of cells of the bone marrow spicule. The reaction is not seen upon substitution of carbodiimide for I, omission of II, or its substitution by RNase or trypsin.

L9 ANSWER 41 OF 41 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1970:98441 HCAPLUS
DOCUMENT NUMBER: 72:98441
TITLE: Nucleases activity in different segments of the **human digestive** tube compared to the incidence of carcinomas (histochemical study)
AUTHOR(S): Fort, L.; Taper, H. S.; Brucher, J. M.
CORPORATE SOURCE: Univ. Louvain, Louvain, Belg.
SOURCE: Histochemie (1969), 20(2), 150-8
CODEN: HICHAU; ISSN: 0018-2222
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Alkaline DNase (I) activity was detected in the basal layer of the squamous epithelium in the esophagus. In the surface epithelium, in the pits and the glands of stomach, I could not be detected. In the duodenum, jejunum, and ileum, I activity was intense. I activity was very weak in the epithelial cells of the colon, sigmoid, rectum, and appendix. The distribution of alkaline RNase was similar to I. Acid **DNase** (**II**) activity was shown in the squamous epithelium of the esophagus. In the stomach, II activity was generally weak. In the duodenum, jejunum, and ileum, II activity was generally intense. II

activity was weak in the colon, sigmoid, rectum, and appendix. Acid RNase had practically the same histochem. pattern as II. The high activity of I and II in the mucosal epithelium coincided with low carcinoma incidence and vice versa.

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=> d his

     (FILE 'HOME' ENTERED AT 09:06:25 ON 04 NOV 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004
L1          1382 S "DEOXYRIBONUCLEASE II"
L2           377 S L1 AND (HUMAN OR MURINE)
L3       3858984 S BETA
L4            36 S L2 AND L3
L5            27 DUP REM L4 (9 DUPLICATES REMOVED)
L6       1714908 S DIGEST?
L7           348 S L1 AND L6
L8            76 S L7 AND (HUMAN OR MURINE)
L9            41 DUP REM L8 (35 DUPLICATES REMOVED)

=> e eastman a r/au
E1             6     EASTMAN A L/AU
E2             3     EASTMAN A M/AU
E3            21 --> EASTMAN A R/AU
E4             2     EASTMAN A S/AU
E5            39     EASTMAN A Y/AU
E6             8     EASTMAN ABAYA R/AU
E7             1     EASTMAN ABRAHAM/AU
E8           177     EASTMAN ALAN/AU
E9            26     EASTMAN ALAN D/AU
E10            4     EASTMAN ALAN DAN/AU
E11            2     EASTMAN ALAN F/AU
E12            1     EASTMAN ALAN R/AU

=> s e3
L10           21 "EASTMAN A R"/AU

=> e krieser r j/au
E1             3     KRIESER J/AU
E2             5     KRIESER R/AU
E3            45 --> KRIESER R J/AU
E4             3     KRIESER R U/AU
E5             1     KRIESER RON/AU
E6             2     KRIESER RONALD/AU
E7            24     KRIESER RONALD J/AU
E8             4     KRIESER RONALD JOE/AU
E9             1     KRIESER T P/AU
E10           15     KRIESER U R/AU
E11            1     KRIESER V R/AU
E12            1     KRIESGMAN J/AU

=> s e3-e8
L11           79 ("KRIESER R J"/AU OR "KRIESER R U"/AU OR "KRIESER RON"/AU OR
                 "KRIESER RONALD"/AU OR "KRIESER RONALD J"/AU OR "KRIESER RONALD
                 JOE"/AU)

=> s l10 or l11
L12           99 L10 OR L11

=> d his

     (FILE 'HOME' ENTERED AT 09:06:25 ON 04 NOV 2004)
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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004
L1          1382 S "DEOXYRIBONUCLEASE II"
L2           377 S L1 AND (HUMAN OR MURINE)
L3       3858984 S BETA
L4            36 S L2 AND L3
L5            27 DUP REM L4 (9 DUPLICATES REMOVED)
L6       1714908 S DIGEST?
L7           348 S L1 AND L6
L8            76 S L7 AND (HUMAN OR MURINE)
L9            41 DUP REM L8 (35 DUPLICATES REMOVED)
                 E EASTMAN A R/AU
L10           21 S E3
                 E KRIESER R J/AU
L11           79 S E3-E8
L12           99 S L10 OR L11

=> s l1 and l12
L13           57 L1 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14           15 DUP REM L13 (42 DUPLICATES REMOVED)

=> d 1-15 ibib ab

L14  ANSWER 1 OF 15     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2003584954     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14664798
TITLE:              Deoxyribonuclease II is a lysosomal
                    barrier to transfection.
AUTHOR:             Howell Dasein Pinto-Gonzalez; Krieser Ronald J;
                    Eastman Alan; Barry Michael A
CORPORATE SOURCE:   Center for Cell and Gene Therapy, Baylor College of
                    Medicine, Houston, Texas 77030, USA.
CONTRACT NUMBER:    GM 56929 (NIGMS)
SOURCE:             Molecular therapy : journal of the American Society of Gene
                    Therapy, (2003 Dec) 8 (6) 957-63.
                    Journal code: 100890581. ISSN: 1525-0016.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200408
ENTRY DATE:         Entered STN: 20031216
                    Last Updated on STN: 20040819
                    Entered Medline: 20040818
AB   DNA delivered in nonviral vectors or as naked DNA must overcome a number
     of extracellular and intracellular barriers to transfection. Since many
     vectors deliver DNA into cells by the endocytic route, DNA degradation by
     lysosomal nucleases has been proposed as a significant barrier to
     transfection, despite the fact that this has not yet been formally
     demonstrated to occur. To test this hypothesis, we have investigated the
     role of deoxyribonuclease II (DNase II), the primary
     acidic endonuclease active in the lysosome, in transfection. Two genetic
     systems were engineered in which mammalian cells either overexpressed
     DNase II or were knocked out for the enzyme. In both models, higher
     levels of DNase II correlated with decreased transfection efficiency by
     nonviral DNA delivery vectors. These data provide direct evidence
     implicating lysosomal DNase II as a barrier to transfection.

L14  ANSWER 2 OF 15     MEDLINE on STN                    DUPLICATE 2
ACCESSION NUMBER:   2003183389     MEDLINE
```

DOCUMENT NUMBER: PubMed ID: 12558498
TITLE: Structural requirements of human DNase II alpha for formation of the active enzyme: the role of the signal peptide, N-glycosylation, and disulphide bridging.
AUTHOR: MacLea Kyle S; **Krieser Ronald J**; Eastman Alan
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Biochemical journal, (2003 May 1) 371 (Pt 3) 867-76.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF047016; GENBANK-AF274571
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20030419
Last Updated on STN: 20030529
Entered Medline: 20030528

AB DNase II alpha (EC 3.1.22.1) is an endonuclease, which is active at low pH, that cleaves double-stranded DNA to short 3'-phosphoryl oligonucleotides. Although its biochemistry is well understood, its structure-activity relationship has been largely unexamined. Recently, we demonstrated that active DNase II alpha consists of one contiguous polypeptide, heavily glycosylated, and containing at least one intrachain disulphide linkage [MacLea, Krieser and Eastman (2002) Biochem. Biophys. Res. Commun. 292, 415-421]. The present paper describes further work to examine the elements of DNase II alpha protein required for activity. Truncated forms and site-specific mutants were expressed in DNase II alpha-null mouse cells. Results indicate that the signal-peptide leader sequence is required for correct glycosylation and that N-glycosylation is important for formation of the active enzyme. Despite this, enzymic deglycosylation of wild-type protein with peptide N-glycosidase F reveals that glycosylation is not intrinsically required for DNase activity. DNase II alpha contains six evolutionarily conserved cysteine residues, and mutations in any one of these cysteines completely ablated enzymic activity, consistent with the importance of disulphide bridging in maintaining correct protein structure. We also demonstrate that a mutant form of DNase II alpha that lacks the purported active-site His(295) can still bind DNA, indicating that this histidine residue is not simply involved in DNA binding, but may have a direct role in catalysis. These results provide a more complete model of the DNase II alpha protein structure, which is important for three-dimensional structural analysis and for production of DNase II alpha as a potential protein therapeutic for cystic fibrosis or other disorders.

L14 ANSWER 3 OF 15 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003082972 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12594037
TITLE: A family history of **deoxyribonuclease II** : surprises from Trichinella spiralis and Burkholderia pseudomallei.
AUTHOR: MacLea Kyle S; **Krieser Ronald J**; Eastman Alan
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755, USA.
CONTRACT NUMBER: CA 23108 (NCI)
CA 50224 (NCI)
SOURCE: Gene, (2003 Feb 13) 305 (1) 1-12.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 20030221
Last Updated on STN: 20030426
Entered Medline: 20030425

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acidic endonuclease found in lysosomes and nuclei, and it is also secreted. Though its Caenorhabditis elegans homolog, NUC-1, is required for digesting DNA of apoptotic cell corpses and dietary DNA, it is not required for viability. However, DNase IIalpha is required in mice for correct development and viability, because undigested cell corpses lead to lesions throughout the body. Recently, we showed that, in contrast to previous reports, active DNase IIalpha consists of one contiguous polypeptide. To better analyze DNase II protein structure and determine residues important for activity, extensive database searches were conducted to find distantly related family members. We report 29 new partial or complete homologs from 21 species. Four homologs with differences at the purported active site histidine residue were detected in the parasitic nematodes Trichinella spiralis and Trichinella pseudospiralis. When these mutations were reconstructed in human DNase IIalpha, the expressed proteins were inactive. DNase II homologs were also identified in non-metazoan species. In particular, the slime-mold Dictyostelium, the protozoan Trichomonas vaginalis, and the bacterium Burkholderia pseudomallei all contain sequences with significant similarity and identity to previously cloned DNase II family members. We report an analysis of their sequences and implications for DNase II protein structure and evolution.

L14 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:213745 HCAPLUS
DOCUMENT NUMBER: 136:227991
TITLE: Protein and cDNA sequences of human and mouse **deoxyribonuclease II** isoenzyme sequence homologs
INVENTOR(S): Eastman, Alan Richard; **Krieser, Ronald Joe**
PATENT ASSIGNEE(S): Trustees of Dartmouth College, USA
SOURCE: U.S., 8 pp., Cont.-in-part of U.S. Ser. No. 541,840.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6358723	B1	20020319	US 2000-574942	20000519
WO 2001075082	A1	20011011	WO 2001-US10635	20010402
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
US 2002028495	A1	20020307	US 2001-949434	20010907
US 6767997	B2	20040727		

PRIORITY APPLN. INFO.: US 2000-541840 A2 20000403
US 2000-574942 A 20000519

AB The present invention provides protein cDNA sequences of novel **DNase II** isoenzyme sequence homologs as well as vectors comprising the cDNA sequences. The invention further discloses that human **DNase II** isoenzyme gene maps on chromosome 1p22. The invention also relates to antibodies against this protein and antisense agents targeted to a cDNA or corresponding mRNA encoding **DNase II** isoforms. In addition, methods of identifying and using modulators of **DNase II** isoform activity are described.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 5 OF 15 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2002424482 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12181746
TITLE: Deoxyribonuclease IIalpha is required during the phagocytic phase of apoptosis and its loss causes perinatal lethality.
AUTHOR: **Krieser R J**; MacLea K S; Longnecker D S; Fields J L; Fiering S; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Cell death and differentiation, (2002 Sep) 9 (9) 956-62.
Journal code: 9437445. ISSN: 1350-9047.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20020816
Last Updated on STN: 20030208
Entered Medline: 20030207

AB Deoxyribonuclease IIalpha (DNase IIalpha) is one of many endonucleases implicated in DNA digestion during apoptosis. We produced mice with targeted disruption of DNase IIalpha and defined its role in apoptosis. Mice deleted for DNase IIalpha die at birth with many tissues exhibiting large DNA-containing bodies that result from engulfed but undigested cell corpses. These DNA-containing bodies are pronounced in the liver where fetal definitive erythropoiesis occurs and extruded nuclei are degraded. They are found between the digits, where apoptosis occurs, and in many other regions of the embryo. Defects in the diaphragm appear to cause death of the mice due to asphyxiation. The DNA in these bodies contains 3'-hydroxyl ends and therefore stain positive in the TUNEL assay. In addition, numerous unengulfed TUNEL-positive cells are observed throughout the embryo. Apoptotic cells are normally cleared rapidly from a tissue; hence the persistence of the DNA-containing bodies and TUNEL-positive cells identifies sites where apoptosis occurs during development. These results demonstrate that DNase IIalpha is not required for the generation of the characteristic DNA fragmentation that occurs during apoptosis but is required for degrading DNA of dying cells and this function is necessary for proper fetal development.

L14 ANSWER 6 OF 15 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002174344 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11906178
TITLE: Revised structure of the active form of human deoxyribonuclease IIalpha.
AUTHOR: MacLea Kyle S; **Krieser Ronald J**; Eastman Alan
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Biochemical and biophysical research communications, (2002 Mar 29) 292 (2) 415-21.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020322
Last Updated on STN: 20020507
Entered Medline: 20020506

AB Deoxyribonuclease IIalpha (DNase IIalpha) is an acid endonuclease found in lysosomes, nuclei, and various secretions. Murine DNase IIalpha is required for digesting the DNA of apoptotic cells after phagocytosis and

for correct development and viability. DNase IIalpha purified from porcine spleen was previously shown to contain three peptides, two of which were thiol crosslinked, all derived by processing of a single polypeptide. Commercial bovine protein is consistent with this structure. However, screening of 18 human cell lines failed to demonstrate this processing, rather a 45 kDa protein was consistently observed. Incubation of cells with the N-glycosylation inhibitor tunicamycin resulted in a 37 kDa protein, which is close to the predicted formula weight. The protein also contains at least one thiol crosslink. Similar results were obtained with overexpressed DNase IIalpha. These results suggest that active DNase IIalpha consists of one contiguous polypeptide. We suggest the previous structure reflects proteolysis during protein purification.

L14 ANSWER 7 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 6
ACCESSION NUMBER: 2002-03382 BIOTECHDS
TITLE: New cDNA encoding a **deoxyribonuclease-II** -beta enzyme useful for degrading DNA present in the mucous plugs in the lungs of cystic fibrosis patients; recombinant DNA-ase-II-beta production and isolation useful for cystic fibrosis therapy and drug screening
AUTHOR: **Eastman A R; Krieser R J**
PATENT ASSIGNEE: Dartmouth-Coll.
LOCATION: Hanover, NH, USA.
PATENT INFO: WO 2001075082 11 Oct 2001
APPLICATION INFO: WO 2001-US10635 2 Apr 2001
PRIORITY INFO: US 2000-574942 19 May 2000; US 2000-541840 3 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-662972 [76]
AB A cDNA encoding a DNA-ase-II-beta enzyme, is new. Also claimed are: a vector comprising the claimed cDNA; an isolated and purified DNA-ase-II-beta enzyme; an antibody against the DNA-ase-II-beta enzyme; determining DNA-ase-II-beta levels in cells, comprising contacting the cells with the above antibody and detecting binding of the antibody; an antisense oligonucleotide targeted to a DNA or mRNA encoding the DNA-ase-II-beta; inhibiting expression of a DNA-ase-II-beta enzyme in cells, comprising administering the above antisense oligonucleotide; and digesting DNA by contacting it with the DNA-ase-II-beta enzyme. The DNA-ase-II-beta may be useful to digest DNA in the mucous plugs in lungs of cystic fibrosis patients and so reduce their viscosity (disclosed). In an example, the cDNA sequence of DNA-ase-alpha was submitted to the GenBank database and a mouse cDNA EST showing high similarity was identified, purchased and sequenced. Additional EST sequences from human tissues were found that had similarity to this EST but contained incomplete sequences. One sequence was found to contain 932bp of the gene referred to here as DNA-ase-II-beta. (11pp)

L14 ANSWER 8 OF 15 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:354949 BIOSIS
DOCUMENT NUMBER: PREV200100354949
TITLE: **Deoxyribonuclease II** proteins and cDNAS.
AUTHOR(S): Eastman, Alan [Inventor, Reprint author]; **Krieser, Ronald** [Inventor]
CORPORATE SOURCE: Hanover, NH, USA
ASSIGNEE: Trustees of Dartmouth College
PATENT INFORMATION: US 6184034 February 06, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 6, 2001) Vol. 1243, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent

LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention provides cDNAs encoding **deoxyribonuclease II** and isolated, purified **deoxyribonuclease II** proteins. Antibodies against this protein and antisense agents targeted to a cDNA or corresponding mRNA encoding **deoxyribonuclease II** are provided. In addition, methods of identifying and using modulators of **deoxyribonuclease II** activity and apoptosis are described.

L14 ANSWER 9 OF 15 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2001297501 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11376952
TITLE: The cloning, genomic structure, localization, and expression of human deoxyribonuclease IIbeta.
AUTHOR: **Krieser R J**; MacLea K S; Park J P; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Gene, (2001 May 16) 269 (1-2) 205-16.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF274571
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010806
Last Updated on STN: 20010806
Entered Medline: 20010802

AB Acidic endonuclease activity is present in all cells in the body and much of this can be attributed to the previously cloned and ubiquitously expressed **deoxyribonuclease II** (DNase II). Database analysis revealed the existence of expressed sequence tags and genomic segments coding for a protein with considerable homology to DNase II. This report describes the cloning of this cDNA, which we term deoxyribonuclease IIbeta (DNase IIbeta) and comparison of its expression to that of the originally cloned DNase II (now termed DNase IIalpha). The cDNA encodes a 357 amino acid protein. This protein exhibits extensive homology to DNase IIalpha including an amino-terminal signal peptide and a conserved active site, and has many of the regions of identity that are conserved in homologs in other mammals as well as C. elegans and Drosophila. The gene encoding DNase IIbeta has identical splice sites to DNase IIalpha. Human DNase IIbeta is highly expressed in the salivary gland, and at low levels in trachea, lung, prostate, lymph node, and testis, whereas DNase IIalpha is ubiquitously expressed in all tissues. The expression pattern of human DNase IIbeta suggests that it may function primarily as a secreted enzyme. Human saliva was found to contain DNase IIalpha, but after immunodepletion, considerable acid-active endonuclease remained which we presume is DNase IIbeta. We have localized the gene for human DNase IIbeta to chromosome 1p22.3 adjacent (and in opposing orientation) to the human uricase pseudogene. Interestingly, murine DNase IIbeta is highly expressed in the liver. Uricase is also highly expressed in mouse but not human liver and this may explain the difference in expression patterns between human and mouse DNase IIbeta.

L14 ANSWER 10 OF 15 BIOSIS on STN DUPLICATE 8
ACCESSION NUMBER: 2001:245207 BIOSIS
DOCUMENT NUMBER: PREV200100245207
TITLE: Structure and processing of human **deoxyribonuclease II**.

AUTHOR(S): MacLea, Kyle S. [Reprint author]; **Krieser, Ronald J.** [Reprint author]; Eastman, Alan [Reprint author]
CORPORATE SOURCE: Pharmacology/Toxicology, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A202. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 23 May 2001
Last Updated on STN: 19 Feb 2002

AB **Deoxyribonuclease II** (DNase II) is an endonuclease active at acidic pH that is found in lysosomes and in various secretions. It has recently been identified as a homolog of the Caenorhabditis elegans NUC-1 protein that is required for digesting the DNA of apoptotic cells and ingested bacteria. The nonhomologous human DNase I protein is used clinically to digest the viscous sputum of cystic fibrosis (CF) patients. However, only marginal improvement in lung function has been observed, probably because DNase I requires exogenous divalent cations and is inhibited by G-actin, abundant in the sputum. In contrast, DNase II is unaffected by divalent cations or actin and may therefore be a superior therapeutic. We are studying the structure and activity of DNase II to evaluate its potential as a CF mucolytic agent. Purified DNase II from porcine spleen and human liver was previously shown to contain two thiol cross-linked peptides derived by posttranslational processing of a single polypeptide with formula weight of 39.6 kDa. Commercial DNase II contains a peptide that begins at amino acid 108, consistent with this structure. However, screening of several cell types and secretions in this laboratory has failed to demonstrate this processing. Rather, these systems show a 51 kDa protein that is reduced in size to 40 kDa upon incubation of cells with the N-glycosylation inhibitor tunicamycin. The protein contains at least one thiol cross-link. Similar results are obtained upon transient transfection of DNase II into several cell lines. Various truncated forms of the protein remain unglycosylated and inactive. These results suggest that the human DNase II protein is formed from one contiguous polypeptide chain, heavily glycosylated, which requires the signal peptide sequence for proper processing. This knowledge is important for production and evaluation of its therapeutic potential for CF.

L14 ANSWER 11 OF 15 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 2000457895 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10903447
TITLE: **Deoxyribonuclease II**: structure and chromosomal localization of the murine gene, and comparison with the genomic structure of the human and three C. elegans homologs.
AUTHOR: **Krieser R J**; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, 03755, Hanover, NH, USA.
CONTRACT NUMBER: CA23108 (NCI)
CA50224 (NCI)
SOURCE: Gene, (2000 Jul 11) 252 (1-2) 155-62.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF190459; GENBANK-AF220525; GENBANK-AF220526
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20001005

Last Updated on STN: 20001005
Entered Medline: 20000925

AB **Deoxyribonuclease II** (DNase II) has been implicated in diverse functions including degradation of foreign DNA, genomic instability, and in mediating the DNA digestion associated with apoptosis. The production of a mouse deleted for DNase II would clearly help to discriminate these functions. We have cloned and sequenced the mouse gene encoding DNase II. It was found to have a similar intron/exon structure to the human gene, although introns 3 and 5 are considerably shorter. The gene is located on mouse chromosome 8. The order of genes at this locus is mGCDH, mEKLF, mDNase II, mSAST, which is the same order that these genes are found on human chromosome 19. The GenBank database contains incorrect expressed sequence tags (ESTs) for the 3' end of the mouse mRNA. Furthermore, the gene structure of two of the three homologs in C. elegans is also incorrectly predicted in the database. We have established the correct intron/exon structure for these genes and show the conserved sequence and structure of the C. elegans, murine and human genes.

L14 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1999310942 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10381642
TITLE: Cleavage and nuclear translocation of the caspase 3 substrate Rho GDP-dissociation inhibitor, D4-GDI, during apoptosis.
AUTHOR: **Krieser R J**; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: CA09658 (NCI)
CA23108 (NCI)
CA50224 (NCI)
SOURCE: Cell death and differentiation, (1999 May) 6 (5) 412-9.
Journal code: 9437445. ISSN: 1350-9047.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990921
Last Updated on STN: 20000303
Entered Medline: 19990909

AB While investigating endonucleases potentially involved in apoptosis, an antisera was raised to bovine **deoxyribonuclease II**, but it recognized a smaller protein of 26 kDa protein in a variety of cell lines. The 26 kDa protein underwent proteolytic cleavage to 22 kDa concomitantly with DNA digestion in cells induced to undergo apoptosis. Sequencing of the 26 kDa protein identified it as the Rho GDP-dissociation inhibitor D4-GDI. Zinc, okadaic acid, calyculin A, cantharidin, and the caspase inhibitor z-VAD-fmk, all prevented the cleavage of D4-GDI, DNA digestion, and apoptosis. The 26 kDa protein resided in the cytoplasm of undamaged cells, whereas following cleavage, the 22 kDa form translocated to the nucleus. Human D4-GDI, and D4-GDI mutated at the caspase 1 or caspase 3 sites, were expressed in Chinese hamster ovary cells which show no detectable endogenous D4-GDI. Mutation at the caspase 3 site prevented D4-GDI cleavage but did not inhibit apoptosis induced by staurosporine. The cleavage of D4-GDI could lead to activation of Jun N-terminal kinase which has been implicated as an upstream regulator of apoptosis in some systems. However, the results show that the cleavage of D4-GDI and translocation to the nucleus do not impact on the demise of the cell.

L14 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:251285 HCAPLUS
DOCUMENT NUMBER: 128:304813
TITLE: Sequence, detection, and apoptosis-inducing activity of human and bovine **DNase II**

proteins and cDNA
INVENTOR(S): Eastman, Alan; **Krieser, Ronald**
PATENT ASSIGNEE(S): Trustees of Dartmouth College, USA; Eastman, Alan; Krieser, Ronald
SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9816659	A1	19980423	WO 1997-US18262	19971009
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6184034	B1	20010206	US 1999-147915	19990323
PRIORITY APPLN. INFO.:			US 1996-28539P P	19961015
			WO 1997-US18262 W	19971009

AB The present invention provides cDNAs encoding **DNase II** and isolated, purified **DNase II** proteins. Antibodies against this protein and antisense agents targeted to a cDNA or corresponding mRNA encoding **DNase II** are provided. In addition, methods of identifying and using modulators of **DNase II** activity and apoptosis are described.
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1999030349 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9812984
TITLE: The cloning and expression of human **deoxyribonuclease II**. A possible role in apoptosis.
AUTHOR: **Krieser R J**; Eastman A
CORPORATE SOURCE: Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03655, USA.
CONTRACT NUMBER: CA09658 (NCI)
CA23108 (NCI)
CA50224 (NCI)
SOURCE: Journal of biological chemistry, (1998 Nov 20) 273 (47) 30909-14.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF047016; GENBANK-AF047017
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981221

AB We have previously implicated **deoxyribonuclease II** (DNase II) as an endonuclease responsible for DNA digestion during apoptosis. The full-length human cDNA has now been cloned. The cDNA contains an open reading frame of 1078 bases coding for a 40-kDa protein. This protein is 10 kDa larger than commercially supplied enzyme, which has been proteolytically cleaved at an internal aspartate residue. The gene is located at chromosome 19p13.2, and has no significant homology to other human proteins, but has >30% identity to three predicted genes in Caenorhabditis elegans. To determine whether overexpression of DNase II induces apoptosis in Chinese hamster ovary cells, the cDNA was cotransfected with a plasmid encoding green fluorescent protein. Within 24 h, a significant proportion of green fluorescent protein-positive cells

contained condensed chromatin, whereas vector-only controls remained viable. Considering that DNase II is normally active only at low pH, it was surprising that transfection induced chromatin condensation. To confirm that transfection was not activating another endonuclease, cells were incubated with the caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-(O-methyl)-fluoromethylketone; this failed to inhibit chromatin condensation induced by DNase II. These results demonstrate that DNase II acts downstream of caspase activation and that it may be activated by an as yet unknown mechanism to induce DNA digestion during apoptosis.

L14 ANSWER 15 OF 15 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1998:197800 BIOSIS
DOCUMENT NUMBER: PREV199800197800
TITLE: Cloning and expression of **deoxyribonuclease II**: A possible role in apoptosis.
AUTHOR(S): **Krieser, R. J.**; Eastman, A.
CORPORATE SOURCE: Dep. Pharmacology, Dartmouth Med. Sch., Hanover, NH 03755, USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1998) Vol. 39, pp. 578. print.
Meeting Info.: 89th Annual Meeting of the American Association for Cancer Research. New Orleans, Louisiana, USA. March 28-April 1, 1998. American Association for Cancer Research.
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 May 1998
Last Updated on STN: 12 Aug 1998

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:08:32 ON 04 NOV 2004

L1 1382 S "DEOXYRIBONUCLEASE II"
L2 377 S L1 AND (HUMAN OR MURINE)
L3 3858984 S BETA
L4 36 S L2 AND L3
L5 27 DUP REM L4 (9 DUPLICATES REMOVED)
L6 1714908 S DIGEST?
L7 348 S L1 AND L6
L8 76 S L7 AND (HUMAN OR MURINE)
L9 41 DUP REM L8 (35 DUPLICATES REMOVED)
E EASTMAN A R/AU
L10 21 S E3
E KRIESER R J/AU
L11 79 S E3-E8
L12 99 S L10 OR L11
L13 57 S L1 AND L12
L14 15 DUP REM L13 (42 DUPLICATES REMOVED)